

EXHIBIT 21

Specification Sheet

Element AVITI™ System

Unrivalled combination of cost, quality, and performance that fits any sequencing application at any scale

Highlights

- Multiple run starts daily
- Complete range of flow cells
- Exceptional accuracy with early insight into data quality
- Seamless compatibility with leading assays

Introduction

Next-generation sequencing (NGS) has revolutionized the field of genomics, empowering researchers to confront complex scientific questions with an evolving portfolio of technology and tools. Offering an unprecedented view of DNA, NGS fuels scientific discovery around the globe. Despite these innovations, the cost of benchtop sequencing has remained high, requiring factory-scale throughput to achieve any savings. A compromise on cost is often at the expense of quality and flexibility. Many labs turn to outsourcing, conceding delays in pursuit of lower costs.

To overcome these tradeoffs and drive more science, the Element AVITI System reimagines the core components of NGS to offer a benchtop platform that grants access to the genomics ecosystem (Figure 1). Delivering flexible throughput at exceptionally low cost, the AVITI System saves time and resources without the need to batch or accept lesser quality. Avidity Sequencing™ forms the core of a disruptive design that readily adapts to any application, offering methods that scale from amplicon to whole genome, and from short-read to long.

Scalable experimental design

Whether an experiment needs 2 billion reads per run or only 100 million, the AVITI System enables cost-effective, high-quality sequencing across a broad scale. Multiple sequencing kit configurations from read lengths of 2 x 75 to 2 x 300 and a full range of high-, medium-, and low-outputs calibrate genomic output without sacrificing cost-effectiveness, even at small scales (Table 1). The kits support a range of insert sizes while accommodating unique dual indexes (UDIs) and unique molecular identifiers (UMIs).



Figure 1. An AVITI System dramatically reduces sequencing costs and turnaround times while elevating the benchmark for sequencing data, all in a compact benchtop format that fits into a variety of spaces.

Individually addressable lanes exert more control over samples and timelines, providing the ability to isolate a library pool in a single lane or sequence two library pools on one flow cell without additional sequencing kits.

Rapid Cloudbreak™ chemistry

Cloudbreak chemistry advances the core Avidity Sequencing technology with increased accuracy, efficiency, and speed. In only 38 hours, two 2 x 150 runs with indexing generate ≤ 600 Gb of data and 2 billion reads. These accelerated turnaround times maximize potential sequencing output during a regular workday, allowing daily completion of up to two 2 x 75 runs.

Industry-leading performance

The AVITI System resets expectations on quality scores (Q-scores), at ≤ 300 cycles delivering the most accurate specification available today with $> 90\%$ of bases scoring Q30.¹ A 2 x 300 kit achieves $> 80\%$. Q-scores exceeding Q40 are routine. An assessment of data quality concluded that across all 20–50x coverages, the AVITI System demonstrated higher accuracy compared to legacy sequencing technology. AVITI System data had fewer soft-clipped reads in difficult homopolymer and repeat regions, among other clear advantages.²

Read Length	High Output Kit (Gb/hours) ^a	Medium Output Kit (Gb/hours)	Low Output Kit (Gb/hours)
<i>Read Count</i>	<i>1 billion^b</i>	<i>500 million</i>	<i>250 million</i>
2 x 75	150/24	75/20	Not applicable
2 x 150	300/38	150/31	75/27
<i>Read Count</i>	<i>300 million</i>	<i>100 million</i>	<i>Not applicable</i>
2 x 300	180/60	60/51	Not applicable

^a Individually addressable lanes slightly extend run times and produce the same output. Each lane contributes half the output.

^b Performance metrics, including read counts, are based on sequencing Element-prepared libraries. Actual results might differ based on factors such as library type and preparation.

Table 2. Output specifications for the AVITI System

Innovative sequencing chemistry

The fundamentals of Avidity Sequencing translate into real-world benefits for data quality and value. The chemistry leverages the unique properties of avidites to execute an efficient sequencing reaction that yields highly accurate data.³ A primary driver of this accuracy is a strong signal-to-noise ratio that persists through high polony densities.

At the start of a run, the library hybridizes to surface primers coating the flow cell. Amplification polymerase then binds to the library and primer duplexes, catalyzing rolling circle amplification (RCA) and generating long DNA strands that include copies of the original library. Each strand forms a polony that contains hundreds of copies of the original library. The polonies hybridize to read-specific sequencing primers.

A cycle begins with a sequencing polymerase binding an avidite to a polony and primer duplex, trapping a base-specific avidite to the polony for imaging and forming an extremely tight complex that enables a 100-fold reduction in reagent concentration compared to sequencing-by-synthesis, by extension driving down the cost per sample (Figure 2). After imaging, the avidites are removed and unlabeled nucleotides are incorporated into the sequencing primer to extend the primer by one nucleotide. Another cycle begins.

Amplification advantages

RCA uses only the original strand as a template to avoid magnifying amplification errors. This amplification method also limits the effects of index hopping and optical duplicates:

- Index hopping assigns reads to the wrong sample and is most pronounced on high-throughput systems using non-RCA amplification. RCA avoids incorporating free index primers into polonies and minimizes index hopping on the flow cell.
- Optical duplicates occur when the software attributes sequences from one large polony to two smaller polonies and separately computes the calls. A low rate of optical duplicates—the rate for the AVITI System is < 1%—results in a greater number of usable reads.

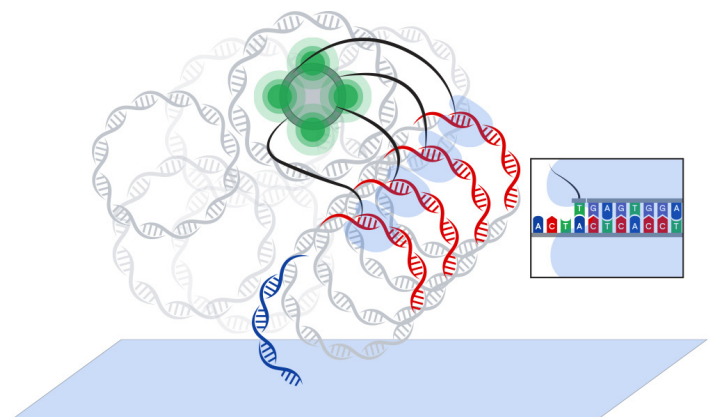


Figure 2. Polymerase binds avidites, trapping them at the incorporation site of template DNA. The avidite arms connect to a core that provides a fluorescent signal for detection. Low-binding surface chemistry makes the signals appear more prominent against a dark backdrop.

Complete NGS solution

The AVITI System grounds an end-to-end NGS workflow that integrates library prep, sequencing, and analysis (Figure 3). Partnerships with a growing range of library prep and analysis companies validate Element solutions and facilitate the transition to the AVITI System. Fixed reagent pricing for the lifetime of the instrument provides assurance for future operational costs and neutralizes batching requirements to expedite results.⁴

Any library prepared with the Element Adept™ Library Compatibility Workflow or Element Elevate™ Library Prep Workflow is compatible with the AVITI System. Both workflows offer robust library prep with broad input requirements and serve as the main entry point for sequencing on the AVITI System. The key difference is methodology: the Adept Workflow adapts existing libraries and the Elevate Workflow prepares libraries from input DNA. 16S LoopSeq™ for AVITI and Amplicon LoopSeq for AVITI provide specialized library prep solutions that are also compatible with the AVITI System.

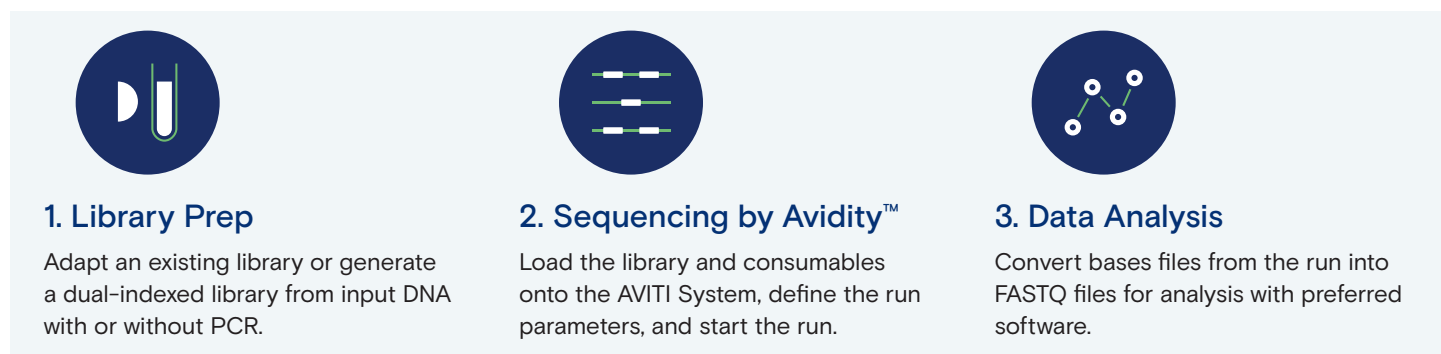


Figure 3. The AVITI System seamlessly integrates genomics resources to offer a sequencing workflow that balances ease of use with the freedom to refine experiments for specific research needs. The AVITI System is compatible with the Adept Workflow, Elevate Workflow, and LoopSeq for AVITI. Bases2Fastq generates FASTQ files.

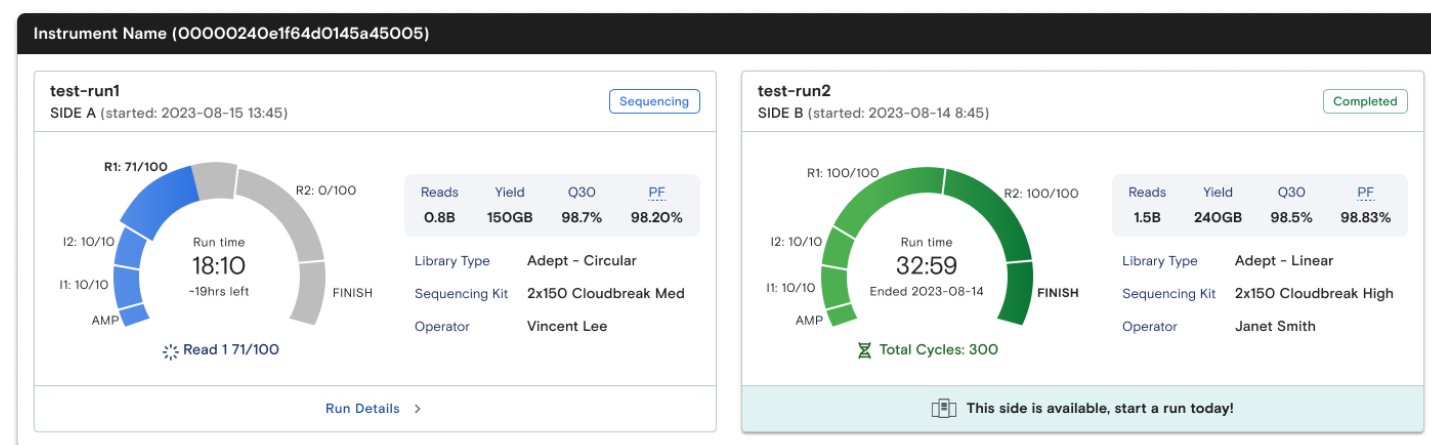


Figure 4. Elembio™ Cloud is an online platform for managing instruments, monitoring runs in real time, and visualizing run metrics to gauge performance.

Adept Workflow for adapted libraries

The Adept Workflow adds Element sequences to linear libraries prepared with a compatible third-party library prep kit. This automation-friendly workflow supports custom primers and allows labs to continue using the same library prep and analysis tools with the AVITI System. Highly accurate quantification optimizes polony density, in turn improving data quality and output.

Optional amplification makes the ends of any incompatible or potentially incompatible library compatible. For an up-to-date list of supported kits, visit go.elembio.link/compatible.

Elevate Workflow for native prep

The Elevate Workflow prepares linear libraries for whole-genome sequencing (WGS). This straightforward workflow integrates with Cloudbreak chemistry to automatically circularize libraries onboard the instrument as part of the run, minimizing hands-on time. A modular kit design enables end-to-end library prep with mechanical or enzymatic fragmentation and the option to integrate Elevate indexes and adapters with a preferred third-party library prep. PCR-free and PCR-plus protocol options round out this flexible WGS solution.

LoopSeq for AVITI bundles Elevate and LoopSeq to generate AVITI-ready libraries with Elevate indexes and adapters. This pairing brings long-read capability to the AVITI System.

Simple and secure data analysis

AVITI Operating Software (AVITI OS) allows you to specify the ideal storage location, keeping genomic data exclusively in your hands.⁵ Guided workflows step through run setup with helpful reminders, menu-style selection of run parameters, and consumable validation. An intuitive user interface guides run setup, run monitoring, and system configuration from the instrument. Elembio Cloud extends AVITI OS capabilities, offering a similarly intuitive view with a rich set of real-time run metrics explorable through a computer or mobile device (Figure 4).

Throughout a run, the software analyzes images and uses the data to call bases and assign Q-scores. These data are packaged into bases files that serve as input for Bases2Fastq Software, which generates FASTQ files for analysis in a preferred application. Features that detect and apply the correct index sequence orientation and automatically identify and trim adapter sequences eliminate guesswork.



Figure 5. A centralized touchscreen monitor (A) simplifies operations. Nests hold two flow cells (B), one for each side, and an LED display (C) communicates the status of a side. The reagents (D) and waste bottles (E) smoothly load and unload from the instrument.

Real-time run QC

An index-first run format sequences the Index 1 and Index 2 reads before the DNA insert, allowing early demultiplexing onboard the instrument for early insight into index assignment metrics, providing confirmation of a high-quality run or sparing the time of a low-quality run. Further downstream, Bases2Fastq detects and applies the correct index sequence orientation for virtually error-proof FASTQ file generation without the guesswork. A related adapter detection feature automatically identifies and trims adapter sequences.

Efficient instrument layout

The AVITI System is a compact benchtop instrument that suits a variety of spaces (Figure 5). Each side of the instrument—side A on the left and side B on the right—is dedicated to one flow cell and operates independently. This dual-sided layout essentially places two systems on the benchtop for the price of one. Moreover, the system ships with accessories designed to minimize waste and facilitate disposal of hazardous reagents.

Dedicated service and support

A dedicated and experienced Element team simplifies service and support and help keep the system operating at peak performance with minimal interruptions. The fully staffed team includes field service engineers to support site prep and installation and verify the system, field application scientists to remove technical barriers and host scientist-to-scientist conversations, and additional engineers and scientists to provide rapid phone and email support.⁶

System sensors measure the performance of key components and send instrument health data to Element. This onboard telemetry rewards labs who partner with Element for proactive system maintenance. Enabling telemetry automatically shares a curated set of metrics that identify potential problems early. The reports are carefully configured to protect sensitive information and do not include any sequencing data. The Element service team cooperates with labs to address any problems quickly and with minimal disruption.

Sequencing at your scale

An alternative model of the full-throughput AVITI System, the AVITI LT runs low- and medium-output sequencing kits to offer low-throughput and budget-friendly access to Avidity Sequencing. If future growth and expanded applications require a broader range of throughputs, labs can easily update the AVITI LT to an AVITI System, which runs all kits. Multi-system labs with high volume can leverage the \$200 Genome Program to sequence at as little as \$200 per genome or \$2 per Gb.

System specifications

Instrument Configuration

Dual flow cells
AVITI Operating Software with a touchscreen display
Ubuntu Core 20.04 LTS operating system

Operating Environment

Temperature: 18–26°C
Elevation: < 2000 m
Sound level: ≤ 62 db at 3.3 ft

Instrument Dimensions

(H x W x D) 29.5 in x 37.6 in x 28.5 in
Weight: 155.1 kg/342 lb

Crate Dimensions

(H x W x D) 48.6 in x 51 in x 35 in
Weight with instrument: 245.9 kg/527 lb

Power Requirements

100–240 VAC at 50/60 Hz, 15 A. 550 W (average)

Summary

The AVITI System reinvents surface chemistry, base detection, and data analysis to offer a flexible and cost-effective sequencing platform that readily supports a variety of NGS applications. From the AVITI LT to the \$200 Genome Program, the AVITI System grows with your needs. Overarching compatibility with standard NGS libraries provides a path to in-house sequencing while integrated and user-friendly software tools streamline operations. Multiple kits at locked prices and abundant software features promote adaptive run setup and analysis to satisfy a spectrum of experiment needs without the demands of batching.

Ordering information

Product	Catalog #
Element AVITI System	880-00001
Element AVITI System LT	880-00003
AVITI 2x75 Sequencing Kit Cloudbreak Medium Output	860-00007
AVITI 2x75 Sequencing Kit Cloudbreak High Output	860-00004
AVITI 2x150 Sequencing Kit Cloudbreak Low Output	860-00005
AVITI 2x150 Sequencing Kit Cloudbreak Medium Output	860-00006
AVITI 2x150 Sequencing Kit Cloudbreak High Output	860-00003
AVITI 2x300 Sequencing Kit Cloudbreak Medium Output	860-00009
AVITI 2x300 Sequencing Kit Cloudbreak High Output	860-00008
Adept Cloudbreak Custom Primer Set	820-00009

To learn more, visit elementbiosciences.com/products/aviti

References

1. Semyon Kruglyak, "Measuring the Accuracy of Element AVITI Sequencing Data," Element Biosciences (blog), July 13, 2022, <https://www.elementbiosciences.com/blog/measuring-accuracy-element-aviti-sequencing-data>.
2. Carroll, Andrew, Alexy Kolesnikov, Daniel E. Cook, et al., "Accurate human genome analysis with Element Avidity sequencing," *bioRxiv* (August 2023): <https://doi.org/10.1101/2023.08.11.553043>.
3. Arslan, Sinan, Francisco J. Garcia, Minghao Guo, et al., "Sequencing by avidity enables high accuracy with low reagent consumption," *Nature Biotechnology* (May 2023): <https://doi.org/10.1038/s41587-023-01750-7>.
4. "Reagent Price Guarantee - Announcement Video," Element Biosciences, accessed February 27, 2023, <https://www.elementbiosciences.com/resources/our-story/our-mission/reagent-price-guarantee-announcement-video>.
5. Element Biosciences, *ElemBio Cloud and Element AVITI System Data Protections White Paper*, July 2023, doc. no. MA-00012.
6. Element Biosciences, *Element AVITI System Site Prep Guide*, October 2022, doc. no. MA-00007.

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EXHIBIT 22



Element
Biosciences

AVITI24™ System

User Guide

FOR USE WITH

AVITI24 System, catalog # 880-00004

AVITI Operating Software v3.3.0 or later

ELEMENT BIOSCIENCES

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Document # MA-00051 Rev. D

April 2025

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CHAPTER 1

System Overview

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Introduction

The AVITI24 System is a multidimensional genomics instrument that integrates state-of-the-art sequencing with cytoprofilng in one benchtop platform. The AVITI24 System measures a diverse array of molecular features for deep multiomic profiling of RNA, protein, and morphology in a single sample in 24 hours.

The AVITI24 System offers flexibility through multiple sequencing kit configurations and Teton™ cytoprofilng kits that support an extensive range of applications. The dual flow cell design enables parallel runs or staggered runs with independent run setup options. AVITI Operating Software (AVITI OS) provides an abundance of additional features to promote adaptive run setup and streamline analysis.

This guide provides an overview of system components, analysis options, maintenance instructions, configuration settings, and safety information for the AVITI24 System.

Site Prep and Safety

Before installation of an AVITI24 System, ensure your site meets the requirements in the *AVITI24 System Site Prep Guide (MA-00052)*. Before operating or maintaining the instrument, review the safety and regulatory information in [Safety and Compliance on page 58](#).

The instrument does not contain any user-serviceable parts. Exterior shells enclose the instrument to protect the operator from laser light exposure and mechanical parts. Software and interlocks prevent exposure to hazards, and using the AVITI24 System in an unspecified manner can compromise these protections.

Warranties and Services

The purchase of an AVITI24 System includes a standard one-year warranty. Element offers supplemental procedures, preventative maintenance service, and annual service plans. For more information, visit elementbiosciences.com/instrument-service-coverage.

System Compatibility

For sequencing runs, the AVITI24 System is compatible with single-strand DNA (ssDNA) libraries prepared with particular library preparation workflows and that use Element sequencing chemistry. For more information on compatibility, see the [Product Compatibility](#) page on the Element website.

To avoid mixing and matching components from different kit configurations and versions, AVITI OS validates the compatibility of the cartridge and flow cell provided in each sequencing or cytoprofilng kit.

Additional Documentation

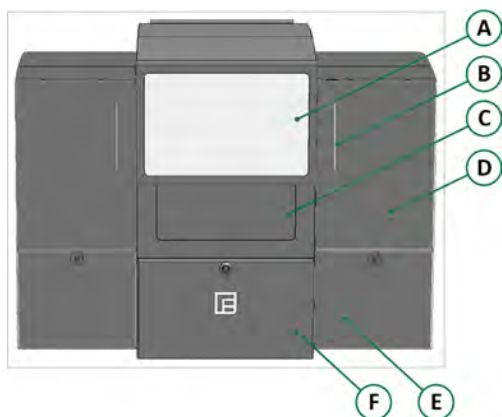
For run preparation and setup instructions for a specific workflow, see the following guides:

- [Cloudbreak Sequencing User Guide \(MA-00058\)](#)
- [Trinity Sequencing User Guide \(MA-00059\)](#)
- [Teton CytoProfiling User Guide \(MA-00053\)](#)

AVITI24 System Components

The instrument is divided into two sides, side A on the left and side B on the right when facing the instrument. Each side operates independently so you can engage one side while the other is in use. Side A and B each include a dedicated pump bay and reagent bay enclosed with bay doors.

Between sides A and B is the glove-compatible touchscreen monitor that displays the AVITI OS interface. Below the monitor is the nest bay and the waste bay. Lighting illuminates the interior of each bay. During a run, AVITI OS locks all doors except the pump bay doors to protect against laser light exposure, mechanical moving parts, and other hazards.



- A Touchscreen monitor
- B Lightbars
- C Nest bay with automated nest door
- D Pump bays hold fluidic pumps
- E Reagent bays hold reagents for each run
- F Waste bay holds waste bottles

CAUTION

Do not place items on top of the instrument or on open doors. The doors can support the weight of run and wash components, but applying extra weight or bumping into an open door can damage the instrument.

Status Lights

The AVITI24 System includes two types of status lights: an interior nest light in front of each nest and an exterior lightbar on each side. The nest light colors indicate flow cell status. The lightbar colors indicate the current process and overall system status. Unless the system is initializing, each lightbar is side-specific.

Nest Light Colors

Color	Status
Blue	The flow cell is present and ready to be unloaded.
Green	The flow cell is properly loaded and ready for priming, sequencing, or washing.
Red	The flow cell is improperly loaded: the lid is open or the nest is empty.
None	The flow cell is present but is not ready to be unloaded.

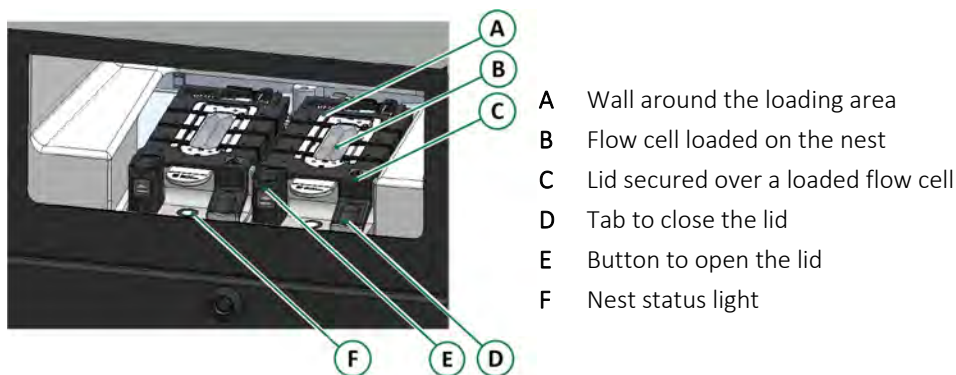
Lightbar Colors

Color	Status
White fade	The system is initializing.
Solid white	The system is initialized and idle.
Solid blue	Run or wash setup is in progress.
Blue fade	The system is priming, sequencing, or washing.
Solid orange	The system experienced a warning. The color changes after the run finishes.
Solid red	The system experienced an error or run failure. The color immediately changes when an error occurs.

Nest Bay

The nest bay includes two nests, one for each side, and each nest holds one flow cell. A hinged flow cell lid secures the flow cell in place. A button on each nest unlatches and opens the hinged lid to a 40° angle. To ensure proper alignment, three silver pins on the loading area fit into three corresponding holes on the flow cell cartridge.

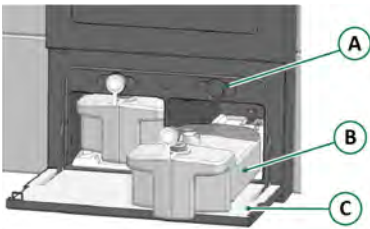
An automated nest door on the middle shell encloses the nest bay. During a run, a camera and four tube lenses above the nest image the flow cell in four channels.



Waste Bay

The waste bay holds two waste bottles, one for each side. Two threaded cap holders above the waste bay secure the tethered transport cap to keep the caps clear of the door.

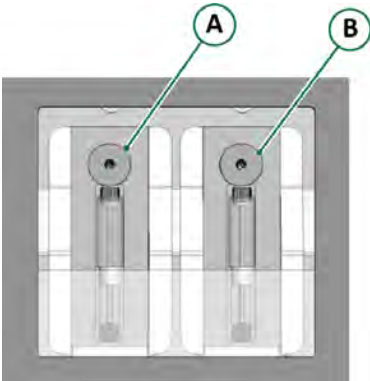
A sealed tray built into the bottom of the waste bay collects spills and leaks and directs liquid to the front of the instrument. During run or wash setup, sensors confirm the waste bottle is present and empty and allows the run or wash to proceed. Another sensor detects spills.



- A Cap holder
- B Waste bottle
- C Open waste bay door

Pump Bays

Each pump bay contains two pumps that control the flow of liquid. The left pump pulls fluid through the left lane of the flow cell and the right pump pulls fluid through the right lane. Keep the pump bay doors, which allow service access, closed during normal operation and maintenance.

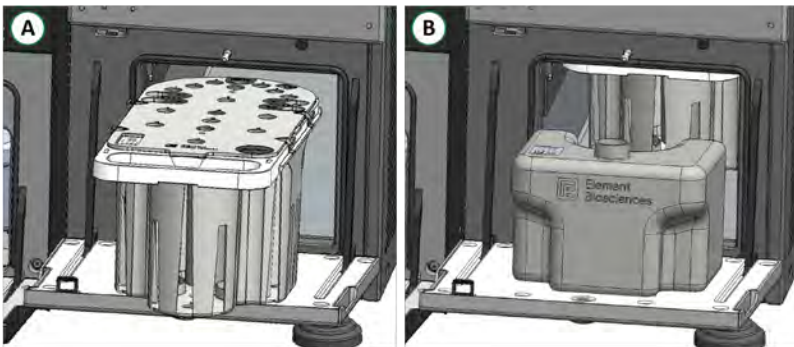


- A Pump controlling the left lane of a flow cell
- B Pump controlling the right lane of a flow cell

Reagent Bays

Each reagent bay holds a buffer bottle and cartridge basket that contains a cartridge or a wash tray, depending on whether the system is sequencing or washing. Keep the reagent bay doors closed to maintain the refrigeration, which chills reagents.

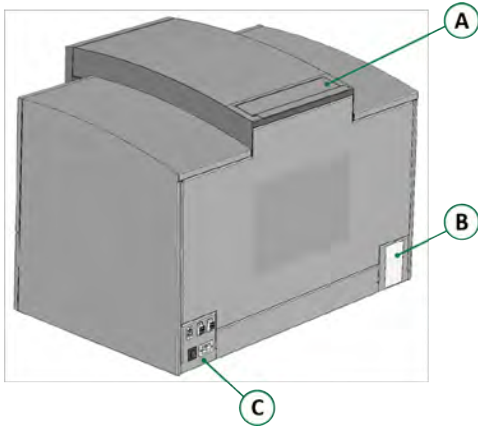
When priming starts, sippers descend into the bay, pierce the foil seals covering the cartridge wells, and aspirate reagents from the bottom of each well. The sippers continue to aspirate reagents throughout the run. Functioning similarly for a wash, the sippers aspirate wash solution instead of reagents.



- A Loading a basket and cartridge
- B Loading a buffer bottle

Back Panel

The back panel includes the air filter tray and input and output (IO) panel. A compliance label displays regulatory symbols for regulatory compliance, the instrument serial number, and electrical specifications. For more information on labeling, compliance, declarations, and certifications, see [Safety and Compliance on page 58](#).



- A Air filter tray
- B Compliance label
- C IO panel

Air Filter Tray

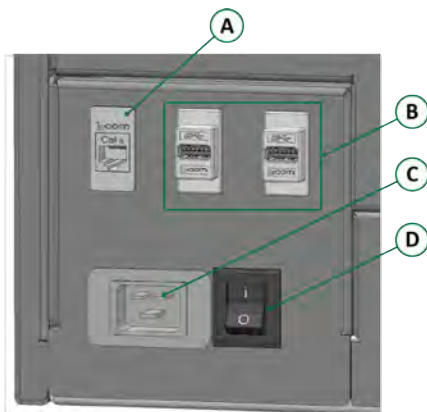
Air enters the instrument through a disposable air filter constructed of pleated paper. The air filter is rated MERV 8, which keeps dust out of the instrument but does not filter smoke or particles < 3 microns. Keeping aerosol and particulate sources away from the instrument extends filter life.

A tray that lifts out of the top of the instrument contains the air filter and facilitates easy replacement. For instructions, see [Replace the Air Filter on page 35](#).

Input and Output Panel

An IO panel on the back of the instrument groups connections and the power switch. A Category 6 (Cat6) Ethernet port connects an Ethernet cable, and a power entry module connects the power cord. When connecting the instrument to power, use only the power cord that Element provides.

The IO panel also includes two USB 3.0 ports to connect a mouse, keyboard, or drive for transferring files. Side B includes a third USB 3.0 port. A USB drive that transfers files to or from the instrument must be in **FAT32 format**.



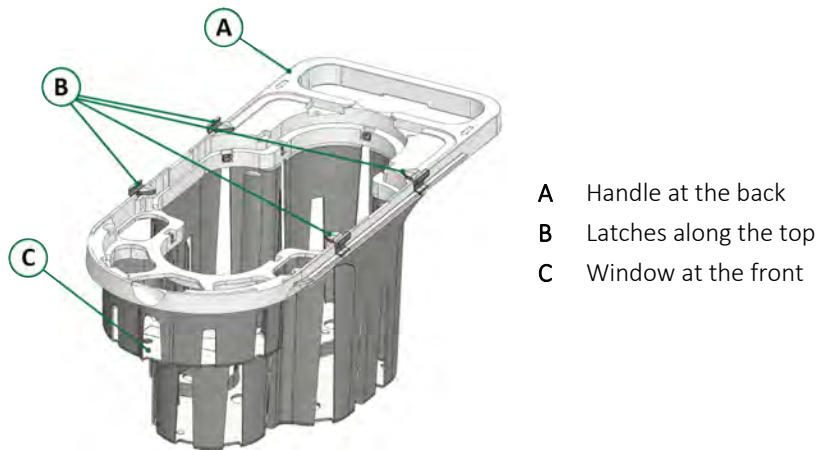
- A Cat6 Ethernet port
- B USB 3.0 ports
- C Power entry module
- D Power switch in the on position

Reusable Accessories

Cartridge baskets, wash trays, and waste bottles support run setup and washes while minimizing waste. These accessories are reusable but require periodic replacement.

Cartridge Basket

The cartridge basket protects the cartridge during a run. The back of the basket extends into a handle with arrows that indicate the loading direction. Clips along the top of the basket secure the cartridge. The curved area under the handle accommodates the buffer bottle, which is loaded into the reagent bay behind the basket. A window at the front of the basket enables library inspection.

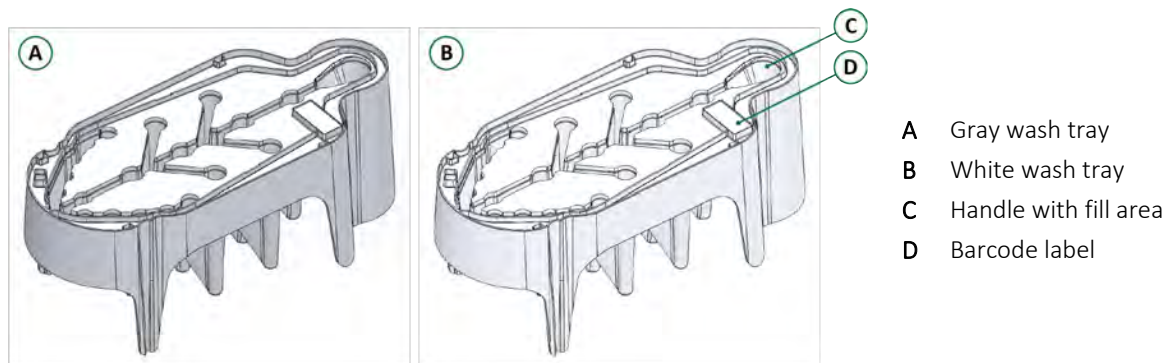


Wash Trays

The AVITI24 System includes two types of wash trays, each dedicated to different wash solutions:

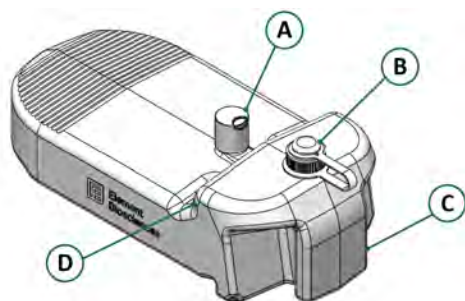
- AVITI Wash Tray 1, Gray, for use with Wash 1 Solution.
- AVITI Wash Tray 2, White, for use with Wash 2 Solution and nuclease-free water.

The back of a wash tray forms a handle with a fill area for adding wash solution. Interior fill lines indicate approximate volumes, and an overflow wall contains any wash solution that exceeds the 800 ml maximum fill volume. Each tray includes a water-proof barcode label for validation purposes.



Waste Bottle

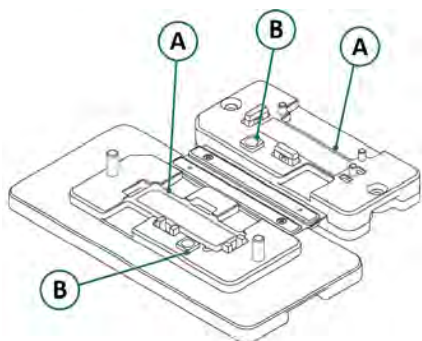
A waste bottle collects spent reagents and library throughout the run. The maximum capacity of 3.2 L per bottle is sufficient to contain all waste from one run. The tethered transport cap seals the bottle during transport. The vent cap improves flow when emptying waste. Ridges on the back of the bottle and a handle at the front facilitate handling.



- A Vent cap
- B Transport cap
- C Handle
- D Thumb indentations

Teton Flow Cell Aligner

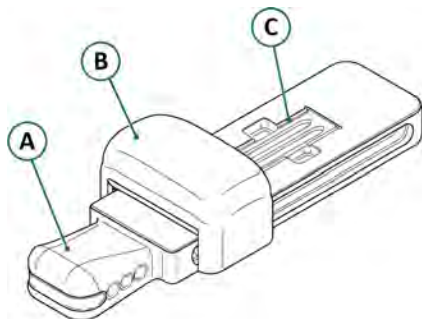
The Teton Flow Cell Aligner is used to combine the prepared sample slide from the slide kit with an adhesive slide from the flow cell assembly kit. After the slides are in position, the hinged fixture is designed to flip the adhesive side against the sample side, affixing the slides together. The affixed slides are then assembled into a flow cell cartridge.



- A Indentation for slide placement
- B Button to release slide holder

Teton Flow Cell Sealer

The Teton Flow Cell Sealer ensures complete adhesion of the sample slide and adhesive slide. An indentation in the tray secures the assembled slides as you slowly push the roller grip forward and back to remove any trapped air.



- A Base grip
- B Roller grip
- C Indentation for slides

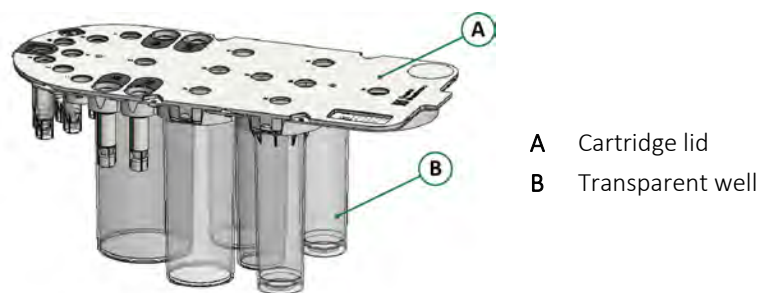
Sequencing Kits

A sequencing kit provides a flow cell, a reagent cartridge, loading buffer, and wash buffer required for one run. The cartridge for each kit supports a specific number of cycles and output levels. Components includes a barcode label for tracking and validation. To ensure the compatibility of run components, see the [Product Compatibility](#) page on the Element website.

Sequencing Cartridge

The reagent cartridge is a collection of reagents and buffers in foil-sealed wells that are packaged in an easy-to-load container. The cartridge lid secures the wells and labels the reagent positions. Each well is transparent to allow visual inspection after thawing. A barcode label enables tracking and validation.

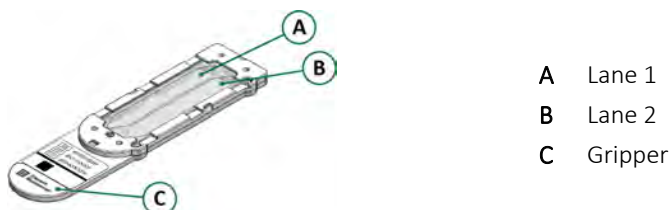
The Library well is reserved for the diluted library. For kits that are compatible with the Individually Addressable Lanes add-on, the AUX well is reserved for a second library. For more information, see [Individually Addressable Lanes on page 19](#).



- A Cartridge lid
- B Transparent well

Sequencing Flow Cell

The flow cell is a two-lane glass substrate encased in a plastic cartridge. The cartridge includes a gripper for safe handling. Proprietary surface chemistry coats the flow cell and enables polony generation and sequencing. Library and reagents enter the flow cell through inlet ports and exit as waste through outlet ports.



- A Lane 1
- B Lane 2
- C Gripper

Loading and Wash Buffers

A sequencing kit includes multiple loading and wash buffers that are packaged separately. Instrument Wash is included in the sequencing cartridge.

Buffer	Packaging	Description
Library Loading Buffer	Tube	The reagent for diluting the libraries to the target loading concentration
AVITI Universal Wash Buffer	Buffer bottle	The reagent that flushes reagents from the flow cell during a run
Instrument Wash	Cartridge	The wash solution for the automatic post-run wash

Cytoprofilng Kits

Kits for a cytoprofilng run are packaged as a reagent kit, a slide kit, and a flow cell assembly kit. Teton reagents support the identification of different cell targets, including cell paint targets, protein and transcript targets, and cell morphology.

- **Teton Reagent Kits**—Each kit includes a Teton reagent cartridge, a buffer bottle, and a Teton reagent kit. One Teton kit provides cytoprofilng and cell paint reagents.
- **Teton Slide Kits**—Slide kits are used to prepare samples for a run or for use with the Teton Optimization Kit for early assessment of your sample.
- **Teton Flow Cell Assembly Kits**—Flow cell assembly kits are required to load one sample slide for a cytoprofilng run.

Reagent Cartridge

The reagent cartridge is a collection of reagents in foil-sealed wells that are packaged in an easy-to-load container. The cartridge lid secures the wells and labels reagent positions. Each well is transparent to allow visual inspection after thawing. A barcode label enables tracking and validation.



Wash Buffers

Cytoprofilng kits include a wash buffer that is packaged separately and labeled with a barcode for tracking and validation purposes. Instrument Wash is included in the reagent cartridge.

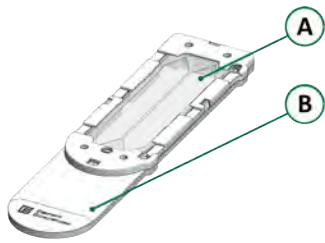
Buffer	Packaging	Description
AVITI Universal Wash Buffer	Buffer bottle	The reagent that flushes reagents from the flow cell during a run
Instrument Wash	Cartridge	The wash solution for the automatic post-run wash

Teton Slide Kit

The Teton workflow requires a Teton Slide Kit for preparing samples directly onto the slide. The slide later becomes part of the Teton flow cell. The slide kit includes a barcode for tracking and validation. For more information, see the *Teton CytoProfiling User Guide* (MA-00053).

Teton Flow Cell Assembly

The Teton flow cell is a combination of two slides affixed together. The affixed slides are assembled into a plastic cartridge with flow cell port gaskets provided in the Teton flow cell assembly kit.



- A Combined slides
- B Assembled flow cell cartridge

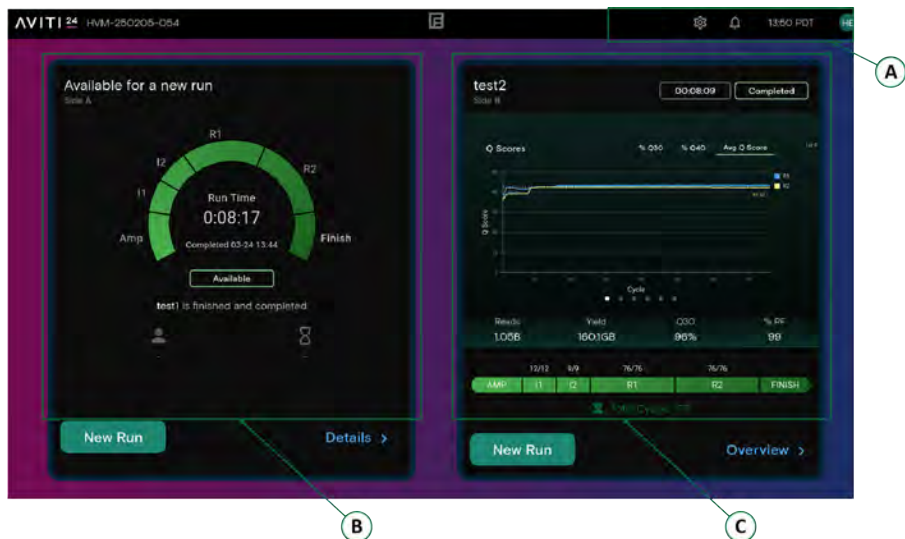
CHAPTER 2

Software and Analysis

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AVITI Operating Software

AVITI OS controls instrument operations during sequencing, analysis, and instrument washes. The Home screen functions as a system dashboard, displaying the status of each side with features to start runs and washes and monitor runs.



- A Taskbar
- B Overview view
- C Details view

Home Screen Views

The Home screen includes buttons that display the following views:

- Overview**—Displays general system status and previous run times for each side, or displays active run or wash information.
- Details**—Displays metrics for an active run. When a run starts, AVITI OS automatically switches to this view.
- History**—Preserves metrics from the last run. When no run or wash is active, this view is available.

Taskbar Icons

A taskbar at the top of the Home screen provides the following icons. The Settings and Notifications icons each open a unique screen. USB Drive and User icons each access additional features and functions.

Icon	Name	Function
	USB Drive	View a list of USB drives that are connected to the instrument and safely disconnect a USB device.
	Settings	View system information and configuration settings. See Settings on page 18 .
	Notifications	Review notifications and perform the indicated action. See Notifications on page 20 .
	User	Open the User menu. Alternatively, this icon displays initials.

Settings

AVITI OS includes configurable and read-only settings that control the instrument profile and system connections. AVITI OS divides the settings among the following tabs:

- **About**—Displays software and instrument information:
 - » AVITI OS version and the last license acceptance date
 - » AVITI24 System name, instrument type, serial number, available local storage, and compute ID
 - » Updates available for system firmware and software




NOTE

Compute ID is a unique code for the integrated circuit that identifies the instrument computer.

- **General**—Controls the system name and displays on-screen keyboard, telemetry, and elevation settings. Also, exports log files from offline systems and resets the air filter time.
- **Network**—Controls network and internet connections for the system. Includes a connectivity indicator.
- **Storage**—Lists storage connections with connectivity indicators and settings for adding and managing storage connections.
- **Add-Ons**—Displays the add-ons enabled on the system and any applicable expiration dates. The tab always appears on offline systems and only appears on online systems with at least one active add-on.
- **User**—Provides password management for offline systems and online systems with local authentication. The tab only appears on applicable systems.



Network Status

The Network tab displays the following icons, which indicate the status of the network connection. An additional Indicator appears on the tab to show internet connectivity.

Icon	Network Status
	Connected
	Local internet only
	Disconnected

Storage Status

The Storage tab displays the following icons, which indicate the status of the storage connections. An additional Indicator appears on the tab to show storage connectivity.

Icon	Storage Status
	At least one storage connection
	No storage connection

Add-Ons

Add-ons enable additional instrument capabilities. The Add-Ons tab displays each add-on available on the instrument. To enable an add-on, contact a sales representative or Element Technical Support.

For online instruments, AVITI OS refreshes the add-on list every 12 hours and when the system restarts. For offline instruments, the installation of add-ons requires additional steps. For more information, see [Install Add-Ons on an Offline System on page 49](#).

Filter Mask

The Filter Mask add-on modifies the cycles used for filtering, which is advantageous for certain applications. Applying the feature causes run output data and on-instrument run metrics to account for the filter mask.

High Output Kits

The High Output Kits add-on enables sequencing with high-output kits.

Individually Addressable Lanes

The Individually Addressable Lanes add-on enables loading one library pool in each lane of a flow cell for sequencing runs. The second library is loaded into the AUX well of the sequencing cartridge.

The add-on is only compatible with sequencing kits that meet the following requirements:

- Cloudbreak™ or Cloudbreak Freestyle™ chemistry
- 2 x 75 or 2 x 150 size
- A high-, medium-, or low-output designation

CAUTION

The Individually Addressable Lanes add-on is **not** compatible with any 2 x 300 size sequencing kits, the Cloudbreak UltraQ™ sequencing kit, or Trinity sequencing kits. The cartridges in these kits reserve the AUX well for other reagents and cannot accommodate a second library.

PMG Shift

Polony map generation (PMG) refers to the process of mapping polonies during a sequencing run. The PMG Shift add-on enables the skipping of up to 20 cycles for compatibility with particular sequencing runs for Adept™ or third-party libraries. Skipped cycles do not affect data output. For more information on the applicability of this add-on, contact Element Technical Support.

ElemBio Catalyst

ElemBio Catalyst™ is a native cloud storage and analysis subscription service within ElemBio Cloud. ElemBio Catalyst allows Element to host and manage cloud storage connections on your behalf. Your data is stored in Amazon Simple Storage Service (Amazon S3) storage buckets that are completely dedicated to you.





To use ElemBio Catalyst, purchase an ElemBio Catalyst subscription or subscribe to a 45-day free trial. If your ElemBio Catalyst storage connection is disabled, an **Expired** badge is displayed for 14 days and the run storage connection cannot be used to upload runs. After 14 days, the ElemBio Catalyst storage connection is no longer visible in AVITI OS. To resubscribe to ElemBio Catalyst, contact Element Technical Support at support@elembio.com. For more information, see the [ElemBio Catalyst documentation](#) in [Online Help](#).

Polony Density

The Polony Density add-on allows users to opt for an increased read output that is prioritized over the highest quality reads and lower error rates. When this add-on is enabled, it is available in the Advanced Run Settings form while you set up a run.



Notifications

Notifications display system messages across three tabs: General, Side A, and Side B. Expand a notification to see the message, date, and time.

Notification	Icon	Description	Action
Success		A run or wash completed successfully.	Acknowledge successful completion.
Information		The software is ready to be updated to a new version.	Acknowledge the update.
Warning		The system requires your attention, but you can continue operation.	Acknowledge the warning and resolve it by the indicated date.
Error		The system has malfunctioned and requires action to proceed.	Follow the onscreen prompt.

Unread Notifications

Notifications include badges that indicate the number of unread messages. Checkboxes mark notifications as read or unread. Marking a notification as read can reset the status lights on that side of the instrument.

Icon	Name	Action
	Mark as read	Mark the selected notifications as read.
	Mark as unread	Mark the selected notifications as unread.

Filtering and Sorting

Notifications include filters with sorting from newest to oldest or oldest to newest.

Filter	Description
All	View all messages on the selected tab.
Read	View only read messages on the selected tab.
Unread	View only unread messages on the selected tab.

Run Start Options

AVITI OS includes the following options for starting a run:

- **Single start**—Set up and start a run on one side of the instrument.
- **Dual start**—Concurrently set up and start runs of the same run type on both sides of the instrument.
- **Flexible start**—Set up and start a run or recovery wash on a side of the instrument while a run is active on the other side.

AVITI OS allows sequencing with different kits on each side. Because the sides share a camera, the setup of one run can increase the duration of the other run.

Flexible Start

Flexible start safely pauses the active run and initiates a run or recovery wash on the other side of the instrument. When setting up the second run, AVITI OS finds a safe pause point before proceeding. While the run is paused, set up and start a run or recovery wash on the other side. The runs on both sides proceed asynchronously. For a flexible start recovery wash, the run on the other side proceeds concurrently.

When you initiate flexible start, AVITI OS indicates the typical wait time for the current run step. Pausing the first run typically takes several minutes but can take as long as ~2 hours, depending on the run step. AVITI OS also includes options to cancel flexible start and resume the active run.

For more information on flexible start wait times, see the run setup instructions in the user guide for your sequencing or cytoprofilng kit.

Wash Setup Screens

Initiating a wash opens a series of wash setup screens that guide you through setting up a maintenance, standby, or recovery wash. Wash setup functions similar to run setup, but closing the door validates the wash tray presence.

Run Setup Screens

When you initiate a run, AVITI OS guides you through a series of run setup screens. Each screen provides a set of steps and indicates run setup progress. AVITI OS unlocks the reagent and waste bay doors at the appropriate steps and prompts the loading of consumables. Closing a door validates the presence of each consumable and scans the consumable barcode. The software presents an alert if consumables are expired. A warning alerts you to expired consumables. Although not supported, AVITI OS allows the run to proceed.

After the step to empty waste and reload the waste bottle, priming starts automatically. Priming prepares reagents for delivery and pumps air and reagents through a used flow cell and the fluidic tubes, preventing contamination between runs.

Run setup steps differ based on the type of kit you are using. For detailed run setup instructions, see [Additional Documentation on page 6](#).

Advanced Run Settings

During run setup, selecting the Advanced Run Settings button displays settings for additional features that let experienced users modify primary analysis and run recipe configurations. Available features depend on your kit selection, run type, and available add-ons.

Feature	Description
Custom Recipes	Tailors a run execution in consultation with Element. A recipe governs the stages of a sequencing run, so custom recipes can impact specifications and increase run times. The setting provides two options for recipes: <ul style="list-style-type: none">• Preloaded recipe: Select a recipe on the instrument, such as the short insert or long insert custom recipes. To ensure run compatibility, contact Element Technical Support.• Uploaded recipe: Element creates an encrypted, custom recipe package as a .rec file, which you upload from a USB. To obtain a .rec file, contact Element Technical Support.
Filter Mask	Sets the mask for the Filter Mask add-on. See Filter Mask on page 19 .
PMG Shift	Sets the number of cycles skipped for the PMG Shift add-on. See PMG Shift on page 19 .
Polony Density	Relaxes certain quality filters to increase the total number of polonies in a run. The setting has two options, with Standard as the default option. The High Density option increases the read output. This feature is also known as Expert Mode HD.

Signing In and Out

Signing in to AVITI OS requires the email address and password for your organization. The first time you sign in to AVITI OS after instrument installation or an update, you must accept the license agreement. A Logout option on the User menu signs you out.

If requested, Element can enable local authentication mode for an online system. This feature assigns a fixed user name and user-defined password to sign in.

Run Manifest

AVITI OS uses a run manifest as an input file that stores run information. The format of the run manifest differs between sequencing and cytoprofilng runs. After a run, AVITI OS provides the run manifest as an output file to support run analysis.

The run manifest uses a comma-separated values (CSV) file format and can be created using a template on the [Resources page](#) of the Element Biosciences website. For more information on creating a run manifest, see the [Run Manifest Documentation](#) in the [Online Help](#).

Sequencing Run Manifest

For sequencing runs, the run manifest includes demultiplexing settings, settings for FASTQ files, and a list of samples with any corresponding index sequences. After a sequencing run, you can use the run manifest that AVITI OS provides to support secondary analysis and Bases2Fastq. Demultiplexing indexed libraries requires preparing a run manifest.

When a sequencing run does not include a run manifest, AVITI OS generates a default run manifest that assigns all reads to one sample during FASTQ file generation.

Demultiplexing indexed libraries is **not possible** with a default run manifest. To use a default run manifest with Bases2Fastq, you must edit the file and create a corrected run manifest that includes sample and index information.

Run Manifest for Sequencing with Individually Addressable Lanes

If you are using the Individually Addressable Lanes add-on, the Lane column in your run manifest must correctly associate samples with both library pools.

- Lane 1 refers to the library pool loaded into the Library well of the sequencing cartridge.
- Lane 2 refers to the library pool loaded into the AUX well of the sequencing cartridge.

For an example run manifest set up for the Individually Addressable Lanes add-on, see [Sample Specification Examples](#) in the [Online Help](#).

Cytoprofilng Run Manifest

For cytoprofilng runs, the run manifest associates each well location with a label. AVITI OS and Cells2Stats can use these labels to refer to each well. Additional optional settings include the cell type and cell diameter.

When a cytoprofilng run does not include a run manifest, AVITI OS generates a default run manifest that uses the names of wells as well labels. The default run manifest does not include any optional settings.

Analysis Overview

Onboard primary analysis depends on the type of run you perform.

- **Sequencing runs:** AVITI OS calls bases, assigns quality scores (Q scores), and generates run metrics. The software extracts and corrects intensities from images to call a base, then assigns a Q score to the base.
- **Cytoprofilng runs:** AVITI OS uses cell paint images to segment cells and generate cell morphology features. The software also extracts and corrects intensities from images to call bases, count targets, and assign targets to cells.

Run Monitoring

During a run, the Details view displays initial estimates for primary-analysis-generated run metrics that monitor overall run health and progress. As the run progresses, metrics appear and regularly update. Sequencing runs that use the Individually Addressable Lanes add-on display metrics and charts for each library pool. The metrics are included in the run output and remain onscreen until you set up a new run.

The metrics and charts for a run depend on your workflow. Additional metrics appear in the charts, which you can cycle through. For more information on the types of metrics and charts that can appear during a run, see [Cytoprofilng Metrics and Charts](#) and [Sequencing Metrics and Charts](#) in the [Online Help](#).

NOTE

To visualize cytoprofilng data with CytoCanvas, execute the Cells2Stats Software after the run completes. To obtain final metrics for a sequencing run, execute the Bases2Fastq Software after the run completes. For more information, see the [Cells2Stats Documentation](#) and [Bases2Fastq Documentation](#) in the [Online Help](#).



Thumbnail Image

The thumbnail image displays a snapshot of the colonies on a tile from the run. For sequencing, the image is from the first cycle of the run. For cytoprofilng, the thumbnail displays the cell membrane and nucleus images from the cell paint batch. If you are performing a sequencing run with the Individually Addressable Lanes add-on and two library pools, AVITI OS displays an image for each pool.

The thumbnail image can serve as a quality check for your run. For sequencing, the image indicates sample density and loading concentration on the flow cell. For cytoprofilng, the image indicates the success of cell fixation.

Run Output and Data Transfer

The output of a run is a run folder. The folder contains files with data for the type of run. A storage connection transfers the run folder from the instrument to your local or cloud storage location. For more information, see [Storage Connections on page 37](#).

Sequencing Run Output

The run folder for a sequencing run contains bases files with genomic data and other run data. Bases files are the primary output of a sequencing run. An AvitiRunStats.json file serves as the source file for run metrics. You can set up the integration of metrics into another system, such as a LIMS interface. For more information on output files, see [Sequencing Run Output Files](#) in the [Online Help](#).

After a sequencing run, use Bases2Fastq to perform demultiplexing and convert the bases files into FASTQ files for secondary analysis with the third-party software of your choice. For more information, see the [Bases2Fastq Documentation](#) in the [Online Help](#).

Cytoprofilng Run Output

The run folder for a cytoprofilng run contains images of cell paint targets, protein and transcript counts per cell, and cell morphology features. The data enable downstream cell analysis and characterization. You can use additional files to regenerate cell statistics and updated cell segmentation masks. For more information on output files, see [Cytoprofilng Run Output Files](#) in the [Online Help](#).

After a cytoprofilng run, if necessary, you can use Cells2Stats to support cell resegmentation and additional analysis of your cell samples. For more information, see the [Cells2Stats Documentation](#) in the [Online Help](#).

Local Disk Storage

Because the system software transfers runs to off-instrument storage locations, local disk storage is intended only for temporary storage. Accordingly, the instrument hard drive has sufficient space to store at least two runs and start an additional two runs. When you initiate run setup, AVITI OS checks whether the system has sufficient space to support the run. If AVITI OS indicates that the system does not have sufficient space, contact Element Technical Support.

Telemetry

Separate from the transfer of genomic data to your storage location, **which Element cannot access**, telemetry sends instrument health data to Element. These data help support maintenance and troubleshooting and do not include any confidential information.

Telemetry is limited to the following data:

- **Software metrics**—Software and firmware versions, CPU and memory metrics, and the instrument serial number, ID, and name. These metrics are communicated as part of regular telemetry events.
- **Hardware metrics**—Data on motors, fans, lasers, and other instrument hardware, which helps Element understand the probable condition of select hardware components.
- **System logs**—Routine logs the system generates when idle or running. The logs include power cycle times, errors, internal communications, and the status of internal services.
- **Primary analysis metrics**—Run metrics, including data for Q30 scores, error rates, cell confluency, cell and target counts, expression levels, and index assignment metrics. Index assignment and other data exclude sample names.
- **Run information**—Data communicated for a run, including run name and ID, run side, run start and end dates and times, run type (sequencing, cytoprofilng, or washing), consumable information, and the number of cycles per read or batch. The data exclude run descriptions.

- **Run logs**—Run-specific information from a subset of system logs. Data include recipe execution, the timing of run steps, and communications between software, firmware, and hardware.

ElemBio™ Cloud

ElemBio Cloud is a central online platform that provides real-time remote run monitoring, data analysis, and account management for Element instruments, including AVITI24 Systems. Any system in online mode automatically connects to the platform. ElemBio Cloud allows you to connect to cloud service providers for data storage and initiate data analysis automation through flows.

You can access ElemBio Cloud on a computer or mobile device to support your organization from anywhere. For more information, see the [ElemBio Cloud Documentation](#) in the [Online Help](#).

ElemBio Cloud Metadata

By default, AVITI OS sends the following metadata to a secure and customer-specific ElemBio Cloud database:

- Run description
- Sample names from the run manifest, if applicable

Metadata populate the run monitoring pages in ElemBio Cloud, which is separate from the telemetry database. Therefore, telemetry does not collect metadata. If you prefer to keep metadata on the instrument, contact Element Technical Support and request Restrict Metadata mode. When the mode is enabled, a lock appears on the run description in ElemBio Cloud and sample names are masked as numbers. The numbering reflects the order of samples in the run manifest.

CHAPTER 3

Maintenance

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Maintenance Schedule

AVITI OS on the AVITI24 instrument requires a maintenance wash every 7 days. The software provides a warning 2 days before a maintenance wash is due.

Procedure	Frequency	Purpose
Maintenance wash*	Weekly	Cleans the outside of the sippers and prevents microbial growth and particulate debris from accumulating in the fluidic system.

Element recommends the following maintenance schedule for optimal performance.

Procedure	Frequency	Purpose
Power cycle	Weekly	Reinitializes the system and resets the instrument computer, which helps maintain instrument performance.
Standby wash*	Preparing for an idle period of ≥ 7 days	Prepares one or both sides for an idle period of ≥ 7 days.
Air filter replacement	Every 6–12 months	Ensures proper cooling and continuous operation. The optimum frequency depends on lab cleanliness.
Exterior cleaning	As needed	Wipe the exterior with a damp microfiber cloth and Simple Green. Avoid harsh chemicals and abrasives.

* To perform a wash after stopping a run, see [Stop an Active Run on page 53](#).

For a list of maintenance consumables, see the *AVITI24 System Site Prep Guide (MA-00052)*.

Wash Tray Maintenance

Keep the wash trays in good condition to maximize time between replacements and prevent cross-contamination.

- After each use, discard residual wash solution, rinse the wash tray with nuclease-free water, and air-dry upside down.
- Store clean and dry wash trays upside down. Stack up to two wash trays.

Power Cycle the System

A power cycle resets the instrument computer, safely shutting down and restarting the system to maintain performance or recover from a problem. Turning off the system without a proper power cycle is reserved for emergencies.

1. Select the user menu, and then select **Shut Down**.
2. When prompted, select **Shut Down** again to shut down the instrument computer.
3. Wait for the screen to go blank and a No Signal message to appear.
4. On the IO panel on the back of the instrument, press the power toggle switch to turn off the instrument.



5. Wait **10 seconds** to make sure the system fully shuts down.
6. On the IO panel, press the power toggle switch to turn on the instrument.
—The system initializes and displays the Home screen.—
7. If a USB drive is connected to the instrument, reconnect it:
 - a. In the taskbar, select **USB Drive**, and then select **Eject**.
 - b. Detach the USB drive from the instrument.
 - c. Reconnect the USB drive to the instrument.
—Reconnecting the USB drive allows AVITI OS to detect it after a power cycle.—

Perform a Maintenance Wash

The maintenance wash is a two-part wash that takes a total of ~1.5 hours. Wash 1 cleans the system, removing residual library and carryover. Wash 2 rinses the system, removing residual Wash 1 solution and preparing for the next run. Each wash requires specific volumes of freshly prepared wash solutions.

Prepare Wash Solutions

1. Gather the following materials:
 - » 2 L bottles (2)
 - » 4.00–4.99% sodium hypochlorite
 - » Gray wash tray
 - » Nuclease-free water
 - » Pipette controller
 - » Serological pipettes (2)
 - » Tween 20
 - » Used flow cell
 - » White wash tray

—A used flow cell might already be present on the instrument.—
2. Add 1.5 L nuclease-free water to a new 2 L bottle.
3. Attach a new serological pipette to a pipette controller.
4. Add 37.5 ml 4.00–4.99% sodium hypochlorite to the bottle to prepare 1.54 L ~0.12% sodium hypochlorite.
5. Label the bottle **Wash 1 Solution**.
6. Cap the bottle and invert several times to mix.
7. Set aside Wash 1 Solution at room temperature. Use within the day or discard.
8. Add 1.5 L nuclease-free water to a new 2 L bottle.
9. Attach a new serological pipette to the pipette controller.
10. Add 4.5 ml Tween 20 to the bottle to prepare 1.5 L 0.3% Tween 20.
11. Label the bottle **Wash 2 Solution**.
12. Cap the bottle and invert several times to mix.
13. Set aside Wash 2 Solution at room temperature.

Initiate a Maintenance Wash

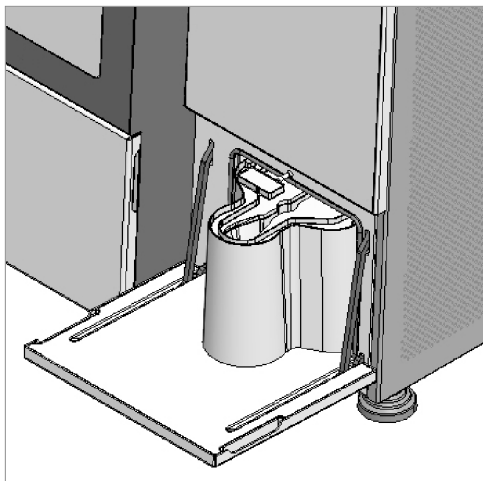
1. On the Home screen, select **New Run**.
2. If AVITI OS prompts that the flow cell is missing, load a *used* flow cell:
 - a. Select **Open Nest**.
 - b. Place the used flow cell onto the nest and close the lid.
 - c. Select **Close Nest**.
3. Select which side to wash:
 - » **Side A**—Set up a maintenance wash on side A.
 - » **Both**—Set up maintenance washes on sides A and B.

» **Side B**—Set up a maintenance wash on side B.

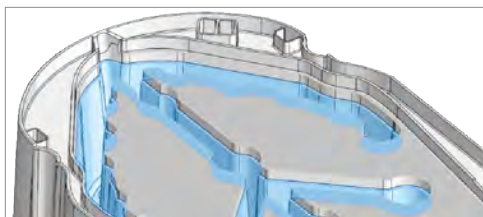
4. Select **Wash**, and then select **Maintenance**.
5. Select **Next** to proceed to the Load Wash 1 screen.

Load Wash 1 Solution

1. Open the reagent bay door.
2. Remove any materials from the reagent bay and set aside.
3. Place a clean, uncovered gray wash tray onto the open door.
4. Slide ~2/3 of the wash tray into the reagent bay, so the barcode edge is about flush with the entrance.



5. Add 590 ml freshly prepared Wash 1 Solution to the fill area, filling the wash tray to slightly above the lower fill line.



6. Slide the wash tray all the way into the reagent bay until it stops and close the reagent bay door.
7. Select **Next** to proceed to the Empty Waste screen.

Empty Waste and Run Wash 1

1. Open the waste bay door.
2. Unscrew the transport cap from the cap holder above the waste bay.
3. Remove the waste bottle from the waste bay and close the transport cap.

CAUTION

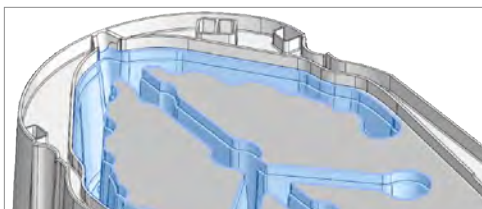
Waste bottle contents are considered hazardous. Dispose of waste according to local, state, and regional laws and regulations.

4. [Optional] Insert a funnel into a waste receptacle. Make sure the funnel is secure.
5. Open the transport cap and the vent cap.
6. Support the waste bottle with both hands and empty the waste:
 - a. Position the bottle over the funnel or waste receptacle.

- If you inserted a funnel, align the handle to the inner edge of the funnel.
 - If you did not insert a funnel, center the handle over the waste receptacle.
 - b. Tip the bottle forward and drain. Invert the bottle and shake to expel all droplets.
 - c. If necessary, wipe liquid off the bottle.
7. Close the vent cap and return the empty waste bottle to the waste bay.
 8. Screw the transport cap onto the cap holder and close the waste bay door.
 9. Select **Next** to open the Run Wash 1 screen and automatically start the wash, which takes ~34 minutes.
 10. During the wash, process the materials removed from the reagent bay:
 - » If you removed a used buffer bottle and cartridge basket, follow the discard instructions in the user guide for the kit.
 - » If you removed a wash tray, follow the guidelines in [Wash Tray Maintenance on page 28](#).
 11. When the wash is complete, select **Next** to proceed to the Load Wash 2 screen.

Load Wash 2 Solution

1. Open the reagent bay door.
2. Remove the gray wash tray from the reagent bay and set aside.
 - Residual liquid in the wash tray is normal.—
3. Place a clean, uncovered white wash tray onto the open door.
4. Slide ~2/3 of the wash tray into the reagent bay, so the barcode edge is about flush with the entrance.
5. Add 660 ml freshly prepared Wash 2 Solution to the fill area, filling the wash tray to slightly above the upper fill line.



6. Slide the wash tray all the way into the reagent bay until it stops and close the reagent bay door.
7. [Optional] Store leftover Wash 2 Solution at 2°C to 8°C for ≤ 2 weeks.

Run Wash 2

1. Select **Next** to open the Run Wash 2 screen and automatically start the wash, which takes ~52 minutes.
2. When the wash is complete, select **Done** to return to the Home screen.
3. Leave all materials in the instrument.
4. Process the gray wash tray from the first wash per [Wash Tray Maintenance on page 28](#).

Perform a Standby Wash

A standby wash takes ~52 minutes and flushes nuclease-free water through the fluidic system, removing any residual Tween 20. When complete, the washed side is idle. Performing a maintenance wash on the idle side ends the idle period and enables sequencing.

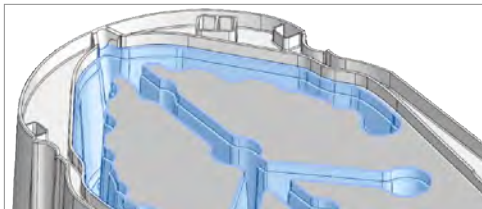
Initiate a Standby Wash

1. Gather the following materials:
 - » Nuclease-free water
 - » Used flow cell
 - » White wash tray

—A used flow cell might already be present on the instrument.—
2. On the Home screen, select **New Run**.
3. If AVITI OS prompts that the flow cell is missing, load a **used** flow cell:
 - a. Select **Open Nest**.
 - b. Place the used flow cell onto the nest and close the lid.
 - c. Select **Close Nest**.
4. Select which side to wash:
 - » **Side A**—Set up a standby wash on side A.
 - » **Both**—Set up standby washes on sides A and B.
 - » **Side B**—Set up a standby wash on side B.
5. Select **Wash**, and then select **Standby**.
6. Select **Next** to proceed to the Load Water screen.

Load Nuclease-Free Water

1. Open the reagent bay door.
2. Remove any materials from the reagent bay and set aside.
3. Place a clean, uncovered white wash tray onto the open door.
4. Slide ~2/3 of the wash tray into the reagent bay, so the barcode edge is about flush with the entrance.
5. Add 660 ml nuclease-free water to the fill area, filling the wash tray to slightly above the upper fill line.



6. Slide the wash tray all the way into the reagent bay until it stops.
7. Close the reagent bay door.
8. Select **Next** to proceed to the Empty Waste screen.

Empty Waste and Run the Standby Wash

1. Open the waste bay door.
2. Unscrew the transport cap from the cap holder above the waste bay.
3. Remove the waste bottle from the waste bay and close the transport cap.

CAUTION

Waste bottle contents are considered hazardous. Dispose of waste according to local, state, and regional laws and regulations.

4. [Optional] Insert a funnel into a waste receptacle. Make sure the funnel is secure.
5. Open the transport cap and the vent cap.
6. Support the waste bottle with both hands and empty the waste:
 - a. Position the bottle over the funnel or waste receptacle.
 - If you inserted a funnel, align the handle to the inner edge of the funnel.
 - If you did not insert a funnel, center the handle over the waste receptacle.
 - b. Tip the bottle forward and drain. Invert the bottle and shake to expel all droplets.
 - c. If necessary, wipe liquid off the bottle.
7. Close the vent cap and return the empty waste bottle to the waste bay.
8. Screw the transport cap onto the cap holder and close the waste bay door.
9. Select **Next** to open the Run Water screen and automatically start the wash.
10. When the wash is complete, select **Next** to proceed to the Remove Tray screen.

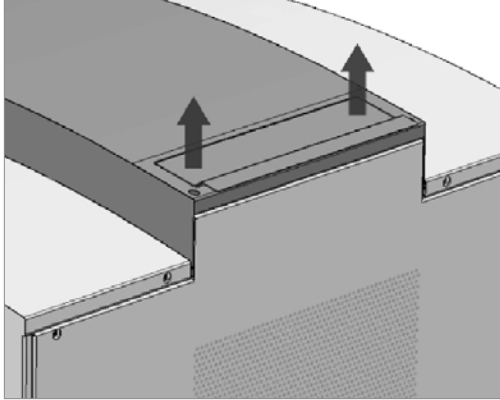
Unload the Wash Tray

1. When prompted, open the reagent bay door and remove the wash tray.
 - Residual liquid in the wash tray is normal.—
2. Close the reagent bay door.
3. Select **Done** to proceed to the Home screen.
4. Leave the flow cell in the nest.
5. Process the materials removed from the reagent bay:
 - » If you removed a used buffer bottle and cartridge basket, follow the discard instructions in the user guide for the kit.
 - » If you removed a wash tray, follow the guidelines in [Wash Tray Maintenance on page 28](#).

Replace the Air Filter

To ensure proper cooling and continuous operation of the system, replace your air filter every 12 months. If your site is located at a high elevation, replace your air filter every 6 months. For more information, see the *AVITI24 System Site Prep Guide (MA-00052)*.

1. If the instrument is performing a run or washing, wait for the run or wash to complete.
2. Select the user menu, and then select **Shut Down**.
3. When prompted, select **Shut Down** again to shut down the instrument computer.
4. Wait for the screen to go blank and a No Signal message to appear.
5. On the IO panel on the back of the instrument, press the power toggle switch to turn off the instrument.
6. Using the flange toward the back of the instrument, lift the air filter tray out of the top.



7. Remove the air filter from the tray and discard.
—The filter might be loose in the tray, which is normal.—
8. Place the tray on a table or benchtop.
9. With the small arrow on the side of the filter pointing up, place the new air filter into the tray.
10. Lower the tray into the instrument. Use the pins to align the tray to the rails and guide entry.
11. On the IO panel, press the power toggle switch to turn on the instrument.
—The system initializes and displays the Home screen.—

CHAPTER 4

System Configuration

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System Connections

The AVITI24 System uses a combination of network, internet, and storage connections to operate. Each system requires a network connection and at least one storage connection. Cloud storage connections, telemetry, over-the-air software updates, and remote support require an internet connection.

Mode	Network Connection	Internet Connection	Storage Connection
Online	Internet	DHCP or static	Cloud or local
	Local	DHCP or static	Local
Offline	Local	None	Local

System Modes

The system mode determines connection options and settings for exporting log files, password protection, and software updates:

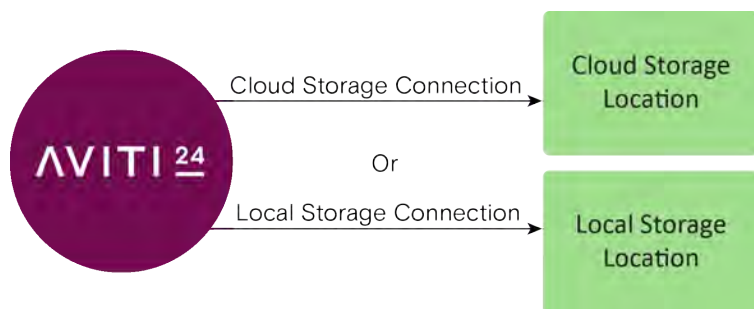
- Online mode connects the system to the internet, which streamlines operations.
- Online local authentication mode operates in online mode but includes local authentication, which avoids network requirements to allowlist Auth0 IP addresses. Only an Element representative can enable this mode.
- Offline mode operates the system without an internet connection. Only an Element representative can enable offline mode.

Storage Connections

A storage connection establishes an off-instrument location that AVITI OS transfers files to. Each run delivers bases files and other run outputs to the default storage location unless you specify a different location during run setup.

AVITI OS supports cloud and local storage connections:

- A cloud storage connection transfers files to a storage location in the cloud.
- A local storage connection transfers files to a storage location on a local network or USB drive.



Storage Connection Requirements

Adding a storage connection requires permissions, network information, and account information that your IT administrator can provide. You must set up cloud storage connections in ElemBio Cloud and fulfill the requirements for the cloud service provider.

For comprehensive storage requirements, see the *AVITI24 System Site Prep Guide (MA-00052)*.

Supported Storage Connections

Cloud storage connections include ElemBio Catalyst, Amazon Web Services (AWS), DNAnexus, and Google Cloud Storage (GCS). For local storage, AVITI OS supports Server Messenger Block (SMB) and USB.

The storage location for a cloud storage connection is a bucket. A connected bucket is available to all systems. Local storage is exclusive to the system.

Cloud Storage

Cloud Storage Connection	Description
ElemBio Catalyst	<ul style="list-style-type: none"> Subscription-based service. Connects the system to an Amazon Simple Storage Service (Amazon S3) bucket that Element creates and operates on your behalf. For more information, see ElemBio Catalyst on page 19. Transfers data using AWS Identity and Access Management (IAM).
AWS	<ul style="list-style-type: none"> Connects the system to an Amazon S3 bucket. Transfers data using secret key authentication through IAM.
DNAnexus	<ul style="list-style-type: none"> Connects the system to a DNAnexus project. Transfers data using an API key for authentication.
GCS	<ul style="list-style-type: none"> Connects the system to a Cloud Storage bucket. Transfers data using secret key authentication through a keyed-hash message authentication code (HMAC).

Local Storage

Local Storage Connection	Description
SMB	<ul style="list-style-type: none"> Connects the system to the server running SMB via a path to a folder. Uses the SMB protocol based on service user authentication to transfer data. Enables import of a run manifest from an SMB storage location during run setup. Supports automatic export of log files from offline systems. Supports Kerberos or NTLMv2 authentication.
USB	<ul style="list-style-type: none"> Transfers data and log files to a USB drive connected to the instrument. Supports automatic and manual export of log files from offline systems. Supports USB-A 3.0 or newer versions and FAT32 or exFAT formats. Must store ≥ 1.6 TB of data, which is sufficient for at least two full runs.

Configure General Settings

General settings include the instrument name setting, the on-screen keyboard setting, file output settings for instrument priming and wash runs, air filter time resetting, and read-only settings that control the instrument profile. For offline systems, general settings also include features to export log files. For instructions, see [Exporting Log Files on page 46](#).

Name the Instrument

1. On the taskbar, select **Settings**.
2. Select the **General** tab, and then select **Edit**.
3. Enter a preferred name consisting of 1–20 alphanumeric characters, hyphens (-), and underscores (_) to identify the instrument.
—The default name is the serial number, field-programmable gate array (FPGA) ID, or Unnamed Instrument.—
4. Select **Save** to apply the name.

Configure On-Screen Keyboard

1. On the taskbar, select **Settings**.
2. Select the **General** tab.
3. Select the **Show on-screen keyboard** toggle to enable or disable the on-screen keyboard for text-entry fields.

Configure File Output Settings

1. On the taskbar, select **Settings**.
2. Select the **General** tab.
3. Select the **Save prime output to storage** toggle to enable or disable the output files for instrument priming.
4. Select the **Save wash run output to storage** toggle to enable or disable the output files for a wash run.
—When you enable the setting, AVITI OS requires you to configure a storage connection before you start a wash.—

Review Read-Only Settings

1. On the taskbar, select **Settings**.
2. Select the **General** tab.
3. Review the following read-only settings. To change a setting, contact Element Technical Support.

Setting	Default	Description
High Elevation	Disabled	Calibrates the system to operate at a high elevation
Offline Mode	Disabled	Prevents an internet connection

Reset the Air Filter Time

1. On the taskbar, select **Settings**.
2. Select the **General** tab.
3. From the Reset Air Filter Time setting, select **Reset**.
4. When prompted, select **Reset** again to confirm that you have replaced your air filter. The timer will be reset.

Connect to the Network

Network settings connect the system to your network via a Dynamic Host Configuration Protocol (DHCP) or a static IP address. When the system is connected to an Ethernet port, AVITI OS automatically connects to a DHCP server and autopopulates the network settings. Alternatively, you can assign a static IP address and manually configure the network settings.

Select a DHCP Server

1.

On the taskbar, select **Settings**.
2.

Select the **Network** tab.
3.

In the drop-down menu, select **Automatic (DHCP)**.
—AVITI OS assigns a dynamic IP address and all other network settings.—

Assign a Static IP Address

1.

On the taskbar, select **Settings**.
2.

Select the **Network** tab.
3.

In the drop-down menu, select **Manual**.
—AVITI OS assigns a unique and permanent IP address.—
4.

Select **Edit**, and then configure the following network settings.

Setting	Example	Description
IP Address	11.2.34.178	The destination IP address
Gateway	11.2.34.177	The IP address of the gateway computer that manages network communications
Subnet Mask	11.2.34.176	The subnet mask that separates the IP address into host and network addresses
Name Server IP(s)	ngs-1.yourlab.com	The names of up to four Domain Name System (DNS) servers that provide IP addresses

—Two additional network settings, Host name and Mandatory Access Control (MAC) address, are read-only.—

5.

Select **Save** to apply the settings and connect to the network.

Add Storage Connections

The Storage tab lists storage connections for the system, including available storage for each local storage connection. An Element representative adds the first storage connection at installation. After installation, you can add an unlimited number of additional storage connections.

AVITI OS only lets you add local storage connections. To add a cloud storage connection, access ElemBio Cloud. For more information, see [Storage Connections](#) in the [Online Help](#).

Add an SMB Storage Connection

1. On the taskbar, select **Settings**.
2. Select the **Storage** tab.
3. Select **Add Storage**, and then select **Local File Server (SMB)** as the storage provider.
4. In the Name field, enter a preferred name for the storage connection.
5. Complete the following fields to configure an SMB network storage location for the SMB storage connection.

Field	Instruction
Host	Enter the host network IP address or fully qualified domain name (FQDN). The Kerberos authentication protocol requires an FQDN. <ul style="list-style-type: none"> • Example IP address: 1.222.333.44 • Example FQDN: datapc.elembio.com
Port	Enter a port number for the file transfer service or leave blank to accept the default of port 445.
Workgroup/Domain	Enter the name of the workgroup or domain that the user belongs to. The Kerberos authentication protocol requires you to enter the Kerberos realm name. <ul style="list-style-type: none"> • Example workgroup/domain for Kerberos: elembio.com
Share	Enter the name of the share that makes the directory accessible to SMB.
Path	Enter the path to an existing folder where you want to output data.
User	Enter the user name for the service user.
Password	Enter the password for the service user.

—All fields except Port and Path are required. Certain server configurations require a work group or domain.—

6. In the Temporary Prefix drop-down menu, select **Disabled** or **Enabled**.

—The Temporary Prefix setting appends two underscores to the name of a file (e.g., __ExampleFileName.zip) while in transfer to the SMB location. The prefix disappears when the file transfer finishes.—
7. In the Session Security drop-down menu, select a setting for the level of encryption:
 - » **High (Recommended)**—AVITI OS requests an encrypted connection with the SMB server. This option is the default setting.
 - » **Medium**—The SMB server determines use of an encrypted connection. The server determines if a connection is encrypted or signed.
 - » **Low**—AVITI OS disables extended SMB security negotiation (SPNEGO) for wider compatibility with SMB servers. The SMB server determines if a connection is encrypted or signed.
8. In the File Checksums drop-down menu, select **Disabled** or **Enabled**.

—The File Checksums setting computes the MD5 checksum for each transferred file and lists them in the RunUploaded.json file. You can use this information to verify the integrity of files.—

9. If prompted, select **Confirm** to set the Session Security selection.
10. Select **Save** to add the storage connection.

Add a USB Storage Connection

For a USB storage connection, the instrument supports USB-A 3.0 or newer versions and the FAT32 or exFAT formats. The USB drive must have > 1.6 TB of available storage space, and the USB name can only use alphanumeric characters, hyphens, and underscores.

1. Connect a USB drive to a USB port on the side or back of the instrument.
2. On the taskbar, select **Settings**.
3. Select the **Storage** tab.
4. Select **Add Storage**, and then select **USB Drive** as the storage provider.
5. In the USB Drive drop-down menu, select the USB drive connected to the instrument.
6. In the Name field, enter a preferred name for the storage connection.
7. Select **Save** to add the storage connection.
 - AVITI OS makes sure the USB drive is connected to the instrument and has write permission and sufficient storage.—

Disconnect the USB for a Storage Connection

1. Select **More** for the USB storage connection, and then select **Eject**.
2. Detach the USB drive from the instrument.
3. To reuse the USB after disconnecting, reconnect the device to a USB port.
 - The USB name must remain the same for AVITI OS to identify the storage connection.—

Manage Storage Connections

Storage settings manage storage connections and include setting the default storage connection. Unless you reset the default storage connection, the default storage connection is the first cloud location added to the instrument. If a cloud location does not exist, the default storage connection is the first local network location.

You can verify any storage connection, but only local storage connections can be edited and deleted in AVITI OS. If you must edit a cloud storage connection, access ElemBio Cloud. For more information, see [Storage Connections](#) in the [Online Help](#).

For ElemBio Catalyst, storage connections that have been expired less than 14 days appear as expired in the storage connections list and cannot be selected in the run setup. Storage connections that have been expired more than 14 days do not appear in the storage connections list. To renew your ElemBio Catalyst subscription, contact your sales representative.

Verify a Storage Connection

1. On the taskbar, select **Settings**.
2. Select the **Storage** tab.
3. For the applicable storage connection, select **More**, and then select **Verify Storage**.
4. Wait ~20 seconds for a success message to appear, indicating a valid storage connection.
—AVITI OS indicates that the connection is connected, unverified, or partially verified with a blocked network.—
5. If AVITI OS cannot verify the storage connection, troubleshoot:
 - a. Make sure the storage connection is correctly set up.
 - For an AWS storage connection, check the IAM permissions. See the applicable [JavaScript Object Notation \(JSON\) policy template](#) in the [Online Help](#).
 - For a GCS storage connection, check the role assigned to the HMAC key.
 - For an SMB storage connection, check the permissions associated with the users.
 - For a USB storage connection, make sure the USB is not ejected, and check that the USB name and type are correct. For USB requirements, see [Local Storage on page 38](#).
 - b. If the storage connection is correctly set up, contact Element Technical Support.

Set the Default Storage Connection

1. On the taskbar, select **Settings**.
2. Select the **Storage** tab.
3. For the applicable storage connection, select **More**, and then select **Set as Default**.
4. When prompted, select **Set Default**.

Edit a Local Storage Connection

1. On the taskbar, select **Settings**.
2. Select the **Storage** tab.
3. For the local storage connection you want to edit, select **More**, and then select **Edit**.
—Editing a busy storage connection can affect where run output is stored.—
4. On the Edit Storage Connection screen, edit any of the following fields.

Field	Instruction
Name	Enter a preferred name for the storage connection.
Workgroup/Domain	Enter the name of the workgroup or domain that the user belongs to. The Kerberos authentication protocol requires you to enter the Kerberos realm name. • Example workgroup/domain for Kerberos: elembio.com
User	Enter the user name for the service user.
Password	Enter the password for the service user.
Session Security	Select High (Recommended) , Medium , or Low .

—The Host, Share, Port, Path, Temporary Prefix, and File Checksums fields are read-only. If you must edit these fields, create another storage connection.—

5. Select **Save** to apply the edits and update the storage connection.

Delete a Local Storage Connection

1. On the taskbar, select **Settings**.
2. Select the **Storage** tab.
3. For the local storage connection you want to delete, select **More**, and then select **Delete**.
4. When prompted, select **Delete**.

—AVITI OS does not allow you to delete a busy storage connection.—

Update the Software

AVITI OS checks for new software versions daily and sends a notification when an update is available. The update runs over-the-air and takes 1-2 hours to complete. Make sure that you initiate the update during instrument downtime to avoid disruptions. If an update exceeds 3 hours and you require support, contact ElemBio Support at support@elembio.com.

For offline systems, Element notifies you of an update and provides the files that are needed for a manual update. Manual updates are only available for systems in offline mode. For instructions, see [Perform a Manual Update on page 49](#).

Perform an Over-the-Air Update

1. Make sure that the AVITI24 System is not performing a run or wash.
2. Power cycle the system. For more information, see [Power Cycle the System on page 29](#).
—For AVITI OS versions 3.3.0 or later, if you haven't performed a system power cycle in the previous 7 days, the system prompts you to power cycle before you start the update.—
3. On the taskbar, select **Settings**, and then select **Update Software**.
4. When prompted, select **Update Now** to start the update.
—The system might restart multiple times during the update process.—
5. After the update completes, power cycle the system when prompted.
6. After the system power cycles, select **Notifications** to view a notification that confirms success.
7. If the update is unsuccessful or takes longer than 3 hours, contact Element Technical Support.
—AVITI OS reverts to the previous version so you can continue operation.—

Manage an Offline System

For AVITI24 Systems in offline mode, AVITI OS lets you export log files, password-protect the system, and perform manual software updates. To install add-ons, offline systems require a specific procedure that uses a USB with an add-on key downloaded from ElemBio Cloud. These procedures and features are unique to offline mode and help manage offline systems.

Exporting Log Files

Offline systems support the export of log files using two methods:

- **Automatic export**—Configure AVITI OS to automatically export log files to a local storage location every hour for telemetry purposes. For help connecting exported log files to telemetry, contact Element Technical Support.
- **Manual export**—Export log files to a USB drive as needed to provide troubleshooting resources to Element Technical Support. AVITI OS lets you perform a limited log file export or a full export of log files.
 - » **Limited Log Export**—Export the log files for a selected run. Use to support initial troubleshooting of a run.
 - » **Full Log Export**—Export all log files for a system. Use to support in-depth system and run troubleshooting.

By default, automatic export is disabled and AVITI OS does not export any log files. When exporting log files to a USB drive, a solid-state drive (SSD) offers significant time savings compared to a flash drive.

Enable Automatic Export of Log Files

1. If necessary, add a local storage connection to export log files to. For instructions, see [Add Storage Connections on page 41](#).
2. On the taskbar, select **Settings**.
3. Select the **General** tab, and then select **Set Up Automatic Export**.
4. In the Storage Connection drop-down menu, select a local storage connection.
5. Select **Save** to enable automatic export.
6. Transfer the exported log files to an internet-accessible location for telemetry.
7. Delete transferred files from the storage location.
 - Each automatic export adds log files to the storage location without replacing or removing existing files.—

Disable Automatic Export of Log Files

1. On the taskbar, select **Settings**.
2. Select the **General** tab.
3. Under Export Log Files, select **Disable** to stop automatically exporting log files.

Change the Automatic Export Location

1. On the taskbar, select **Settings**.
2. Select the **General** tab.
3. Under Export Log Files, select **Edit**.
4. In the Storage Connection drop-down menu, select a local storage location to export log files to.
5. Select **Save** to reset the location.

Manually Export Full Log Files

1. Connect a USB drive to a USB port on the side or back of the instrument.

2. On the taskbar, select **Settings**.
3. Select the **General** tab.
4. In the drop-down menu for manual exports, select **Full Log Export**.
5. In the USB Drive drop-down menu, select the USB drive connected to the instrument.
6. Enter an Export Range using the Start Date, End Date, and time fields.
—The Export Range cannot exceed 14 days.—
7. [Optional] Select the **All Day** toggle to remove time fields and export all log files for the dates in the Export Range.
8. Select **Export Logs**.
—AVITI OS exports the log files to the USB drive.—
9. On the taskbar, select **USB Drive**, and then select **Eject** to disconnect the USB drive.
10. Detach the USB drive from the instrument.
11. Upload the log files to the location that Element Technical Support provides.

Manually Export Limited Log Files

1. Connect a USB drive to a USB port on the side or back of the instrument.
2. On the taskbar, select **Settings**.
3. Select the **General** tab.
4. In the drop-down menu for manual exports, select **Limited Log Export**.
5. In the USB Drive drop-down menu, select the USB drive connected to the instrument.
6. In the Export Run drop-down menu, select the run for which you want log files.
 - » Only runs from the last 14 days are available.
 - » If you select an active run, only certain files might be available.
 - » If you attempt to export log files soon after an active run starts, you might receive an error message. Wait until the run progresses further and attempt the export again.
7. Select **Export Logs**.
—AVITI OS exports the log files to the USB drive.—
8. On the taskbar, select **USB Drive**, and then select **Eject** to disconnect the USB drive.
9. Detach the USB drive from the instrument.
10. Upload the log files to the location that Element Technical Support provides.

Manage Passwords

User settings manage passwords for offline systems and online systems with local authentication. Offline systems support setting, changing, resetting, and removing passwords. An online system supports password reset and removal only.

NOTE

Resetting or removing a password requires assistance from Element Technical Support.

Set a Password

1. On the taskbar, select **Settings**.
2. Select the **User** tab.
3. In the Password field, enter a new password.
—The field accepts ≥ 4 alphanumeric and special characters, excluding spaces.—

4. In the Confirm Password field, reenter the new password.
5. Select **Save**.
6. When prompted, select **Yes, Set Password**.

Change the Password

1. On the taskbar, select **Settings**.
2. Select **User**.
3. In the Current Password field, enter the current password.
4. In the Password field, enter a new password.
—The field accepts ≥ 4 alphanumeric and special characters, excluding spaces.—
5. In the Confirm Password field, reenter the new password.
6. Select **Save** to apply the new password.

Reset a Lost Password

1. On the login screen, select **Forgot Password**.
2. Select **Generate** to display a password reset token and the instrument serial number.
3. Contact Element Technical Support and provide the token and serial number.
—Element Technical Support emails you a single-use password reset file.—
4. Save the password reset file at the root level of a USB drive. Do not rename the file or save it in a folder.
5. Connect the USB drive to a USB port on the side or back of the instrument.
6. Select **Next**.
7. Select **Load Reset File** to upload the password reset file, which removes the password from the system.
8. In the Password field, enter a new password.
—The field accepts ≥ 4 alphanumeric and special characters, excluding spaces.—
9. In the Confirm Password field, reenter the new password.
10. Select **Reset Password** to apply the new password and return to the login screen.
11. Sign in to the system using the new password.
12. On the taskbar, select **USB Drive**, and then select **Eject** to disconnect the USB drive.
13. Detach the USB drive from the instrument.
14. Discard the password reset file.

Remove the Password

1. On the taskbar, select **Settings**.
2. Select **User**, and then select **Remove Password**.
3. When prompted, select **Yes, Remove Password**.
4. Select **Generate** to display a password reset token and the instrument serial number.
5. Contact Element Technical Support and provide the token and serial number.
—Element Technical Support emails you a single-use password reset file.—
6. Save the password reset file at the root level of a USB drive. Do not rename the file or save it in a folder.
7. Connect the USB drive to a USB port on the side or back of the instrument.
8. Select **Next**.

9. Select **Load Reset File** to upload the password file, which removes the password from the system.
10. On the taskbar, select **USB Drive**, and then select **Eject** to disconnect the USB drive.
11. Detach the USB drive from the instrument.
12. Discard the password reset file.

Perform a Manual Update

1. Unzip the files that Element provides for the update.
2. Save the files at the root level of an exFAT USB drive with a minimum storage of 12 GB.
3. Connect the USB drive to a USB port on the side or back of the instrument.
4. Make sure the AVITI24 System is not performing a run or wash.
5. Power cycle the system. For more information, see [Power Cycle the System on page 29](#).
—For AVITI OS versions 3.3.0 or later, if you haven't performed a system power cycle in the previous 7 days, the system prompts you to power cycle before you start the update.—
6. On the taskbar, select **Settings**.
7. Under Software Update, in the USB Drive drop-down menu, select the USB drive that contains the update files.
8. When prompted, select **Update Now** to perform the update.
—The system might restart multiple times as it updates.—
9. After the update completes, power cycle the system when prompted.
10. After the system power cycles, select **Notifications** to view a notification that confirms a successful update.
11. If the update is unsuccessful or takes longer than 3 hours, contact Element Technical Support.
—AVITI OS reverts to the previous version so you can continue using the system.—
12. On the taskbar, select **USB Drive**, and then select **Eject** to disconnect the USB drive.
13. Detach the USB drive from the instrument.

Install Add-Ons on an Offline System

Add-ons on offline AVITI24 Systems require an installation procedure using a USB with a key downloaded from ElemBio Cloud. The add-on key file must be located at the root level of the USB. For instructions to download the add-on key, see [Add-Ons](#) in the [Online Help](#).

1. Use a USB port on the side or back of the instrument to connect the USB drive with the add-on key file downloaded from ElemBio Cloud.
2. On the taskbar, select **Settings**.
3. Select the **Add-Ons** tab.
4. In the drop-down menu, select the USB Drive.
5. Select **Upload**.
—AVITI OS uploads the key file, which installs the add-ons. The Add-Ons tab displays the add-ons and expiration dates.—

CHAPTER 5

Troubleshooting

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General Troubleshooting

Error messages communicate hardware or software problems and describe both the problem and resolution. General troubleshooting resolves other problems that can occur during system initialization, run setup, and run progression. If a problem persists, contact Element Technical Support.

A power cycle resolves many common problems. For instructions, see [Power Cycle the System on page 29](#).

Problem	Resolution
After turning on the instrument, the monitor does not display AVITI OS.	Power cycle the system.
The initialization sequence does not complete, and the loading screen persists.	
The software, instrument, keyboard, or mouse stop operating.	
The reagent or waste bay contains liquid.	See Clean Spills and Leaks on page 56 .
Liquid is spilling from the front or bottom of the instrument.	
The nest is wet.	
AVITI OS detects a full waste bottle, but the bottle is empty.	Reload the waste bottle and make sure the waste bay is unobstructed.
The reagent bay contains condensation.	Dry the inside of the reagent bay with a clean, dry microfiber cloth. Clean to the back of the bay, avoiding sensors and cables.
The instrument does not accept a USB device.	Confirm that the USB device meets the following requirements: <ul style="list-style-type: none"> • Contains > 1.6 TB available space. • Uses USB-A 3.0 or a newer version. • Uses the FAT32 or exFAT format. • Uses a name with alphanumeric characters, hyphens, and underscores only.
AVITI OS does not proceed with run setup despite sufficient storage space on the instrument.	Set up a storage connection to use with the run. A storage connection is required for a run, and on-instrument storage is reserved as a backup in case of network disruption.
A run continues after you stop it.	Wait for the run to stop. AVITI OS waits for a safe point to stop the run, which can take several minutes to ~2 hours depending on the run stage.
Network connection is lost in the middle of a run.	Wait for the AVITI24 System to reconnect to the network. Sequencing and cytoprofilng chemistry are not impacted by network disruptions, and the run progresses as expected. After the system reconnects, data transfer resumes. The system has enough local disk storage for two sequencing runs.

Problem	Resolution
Connection to storage location is lost, and the instrument cannot reconnect.	Confirm with your IT department that all necessary ports and URLs in the <i>AVITI24 System Site Prep Guide (MA-00052)</i> are allowlisted.
The run folder is missing data.	Make sure the user interface indicates that the system is uploading and wait for the upload to complete. <ul style="list-style-type: none">• A slow connection delays data transfer.• Data transfer failure prompts a notification.

Cancel Runs and Washes

AVITI OS displays the following buttons for canceling runs and washes:

- **Discard**—Cancels run or wash setup. The button appears when you can discard setup without compromising consumables.
- **Stop**—Appears on the Home screen and cancels an active run. The button is always enabled so you can free the instrument when run parameters are incorrect, data quality is poor, or a hardware problem occurs.

Discard Run Setup

1. On any run setup screen before priming, select **Discard**.
2. When prompted to confirm the discard, select an option:
 - » **Unlock Door A** or **Unlock Door B**—Discard the run and save the cartridge.
 - » **Discard Setup**—Discard the run, delete the run, and return to the Home screen without saving the cartridge.
3. If you unlocked the door, proceed with the remaining steps.
4. Open the reagent bay door and remove the cartridge.
5. Place the cartridge on ice or refrigerate at 2°C to 8°C.
6. Place a clean, uncovered white wash tray onto the open reagent bay door.
7. Slide ~2/3 of the wash tray into the reagent bay, so the barcode edge is about flush with the entrance.
8. Add 660 ml nuclease-free water to the fill area, filling the wash tray to slightly above the upper fill line.
9. Slide the wash tray all the way into the reagent bay until it stops.
10. Close the reagent bay door.
 - AVITI OS deletes the run setup and returns to the Home screen.—
11. Set up a new run and use the cartridge within **4 hours**.

Discard Wash Setup

1. On any wash setup screen, select **Discard**.
2. When prompted to confirm the discard, select **Discard Setup**.
 - AVITI OS deletes the wash setup and returns to the Home screen.—

Stop an Active Run

Stopping an active run is a two-part process: stop the run, then perform a ~60-minute recovery wash to remove residual library from the fluidic system.

CAUTION

Stopping a run is *final*. You cannot resume a stopped run or reuse any of the consumables.

Stop the Run

1. On the applicable side of the Home or Run Details screen, select **Stop**.
2. When prompted, select **Yes, Stop Run**.
 - AVITI OS finishes the current step, terminates the run, and returns to the Home screen.—
3. Proceed to [Prepare Wash 2 Solution on page 54](#) and complete the recovery wash.

Prepare Wash 2 Solution

1. Gather the following materials:
 - » 2 L bottle
 - » Nuclease-free water
 - » Pipette controller
 - » Serological pipette
 - » Tween 20
 - » Used flow cell
 - » White wash tray

—A used flow cell might already be present on the instrument.—
2. Add 1.5 L nuclease-free water to a new 2 L bottle.
3. Attach a new serological pipette to a pipette controller.
4. Add 4.5 ml Tween 20 to the bottle to prepare 1.5 L 0.3% Tween 20.
5. Label the bottle **Wash 2 Solution**.
6. Cap the bottle and invert several times to mix.
7. Set aside Wash 2 Solution at room temperature.

Initiate a Recovery Wash

1. On the Home screen, select **New Run**.
2. If AVITI OS prompts that the flow cell is missing, load a *used* flow cell:
 - a. Select **Open Nest**.
 - b. Place the used flow cell onto the nest and close the lid.
 - c. Select **Close Nest**.
3. Select which side to wash:
 - » **Side A**—Set up a recovery wash on side A.
 - » **Both**—Set up recovery washes on sides A and B.
 - » **Side B**—Set up a recovery wash on side B.
4. Select **Wash**, and then select **Recovery**.
5. Select **Next** to proceed to the Load Wash 2 screen.

Load Wash 2 Solution

1. Open the reagent bay door.
2. Remove the buffer bottle and cartridge basket from the reagent bay. Set aside both materials.
3. Place a clean, uncovered white wash tray onto the open door.
4. Slide ~2/3 of the wash tray into the reagent bay, so the barcode edge is about flush with the entrance.
5. Add 660 ml freshly prepared Wash 2 Solution to the fill area, filling the wash tray to slightly above the upper fill line.
6. Slide the wash tray all the way into the reagent bay until it stops.
7. Close the reagent bay door, and select **Next** to proceed to the Empty Waste screen.
8. [Optional] Store leftover Wash 2 Solution at 2°C to 8°C for ≤ 2 weeks.

Empty Waste and Run Wash 2

1. Open the waste bay door.
2. Unscrew the transport cap from the cap holder above the waste bay.
3. Remove the waste bottle from the waste bay and close the transport cap.

CAUTION

Waste bottle contents are considered hazardous. Dispose of waste according to local, state, and regional laws and regulations.

4. [Optional] Insert a funnel into a waste receptacle. Make sure the funnel is secure.
5. Open the transport cap and the vent cap.
6. Support the waste bottle with both hands and empty the waste:
 - a. Position the bottle over the funnel or waste receptacle.
 - If you inserted a funnel, align the handle to the inner edge of the funnel.
 - If you did not insert a funnel, center the handle over the waste receptacle.
 - b. Tip the bottle forward and drain. Invert the bottle and shake to expel all droplets.
 - c. If necessary, wipe liquid off the bottle.
7. Close the vent cap and return the empty waste bottle to the waste bay.
8. Screw the transport cap onto the cap holder and close the waste bay door.
9. Select **Next** to open the Run Wash 2 screen and automatically start the wash.
10. When the wash is complete, select **Next** to proceed to the Home screen.
11. Discard the cartridge and buffer bottle and wash the basket. See the discard instructions in the user guide for your sequencing or cytoprofilng kit.

Clean Spills and Leaks

Clean the nest, waste bay, or reagent bay to recover from a leak or spill observed when setting up a run or wash. A leak or spill that occurs in the waste bay during a run causes an error and requires cleaning and contacting Element Technical Support.

If the bottom of the instrument is leaking or liquid is spilling from the front of the instrument: shut down and unplug the instrument if doing so is safe and contact Element Technical Support.

Clean the Nest

1. Dampen a microfiber cloth with isopropyl alcohol.
2. Wipe the nest with the damp microfiber cloth and allow to dry.
3. If necessary, use a polyurethane foam-tip swab to clean additional areas around the nest.
4. Resume run or wash setup.

Clean the Reagent Bay

1. Keep the reagent bay door open.
2. Remove any materials from the reagent bay and set aside.
3. Wipe the interior of the reagent bay with a damp microfiber cloth, cleaning to the back of the bay while avoiding sensors and cables.
4. Inspect the exterior of the instrument for any visible fluid. If necessary, wipe with a damp microfiber cloth.
5. Resume run or wash setup.

Clean the Waste Bay

1. Keep the waste bay door open. If the leak occurs during a run, open the door:
 - a. Wait for any runs or washes on the unaffected side to finish.
 - b. On the taskbar, select **Notifications**.
 - c. On the applicable error, select **Unlock Waste Module Door**.
 - d. Open the waste bay door.
2. Unscrew the transport cap from the cap holder on the affected side.
3. Remove the waste bottle from the waste bay and close the transport cap.

CAUTION
Waste droplets might be on the exterior of the waste bottle.
4. Inspect the waste bottle for cracks, holes, and other defects.
5. [Optional] Insert a funnel into a waste receptacle. Make sure the funnel is secure.
6. Open the transport cap and the vent cap.
7. Support the waste bottle with both hands and empty the waste:
 - a. Position the bottle over the funnel or waste receptacle.
 - If you inserted a funnel, align the handle to the inner edge of the funnel.
 - If you did not insert a funnel, center the handle over the waste receptacle.
 - b. Tip the bottle forward and drain. Invert the bottle and shake to expel all droplets.

- c. If necessary, wipe liquid off the bottle.
- 8. Close the vent cap, leave the transport cap open, and set aside the waste bottle.
- 9. Wipe the interior of the waste bay with a damp microfiber cloth.
- 10. Inspect the exterior of the instrument for any visible fluid. If necessary, wipe with a damp microfiber cloth.
- 11. Return the waste bottle to the waste bay.
 - » If the bottle is defective and you have a spare, load the spare.
 - » If the bottle is defective and you do not have a spare, load the defective bottle. Do not use the affected side until the defective waste bottle is replaced.
- A run or wash on either side requires the presence of both bottles.—
- 12. Screw the transport cap onto the cap holder and close the waste bay door.
- 13. Resume run or wash setup. If necessary, set up a new run with new consumables and clean accessories.

CHAPTER 6

Safety and Compliance

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General Safety

Review this chapter before operating or maintaining the AVITI24 System to ensure safe, correct usage. The procedures described in this guide are tested and optimized, so any deviation can compromise results, cause personal injury, or damage the instrument. All personnel operating the instrument must be trained in correct operation and safety.

The AVITI24 System Site Prep Guide (MA-00052) provides delivery information and installation requirements, including instrument specifications, power specifications, and environmental conditions. A field service engineer (FSE) installs the AVITI24 System.

WARNING
Do not attempt to move the instrument, which can result in injury. Only trained Element personnel are qualified to safely move the instrument.

Safety Labels

The following table lists the safety labels affixed to the instrument. The labels identify potential hazards associated with installation, service, and operation. Follow the procedures in this guide as described to avoid interactions that expose you to these hazards.

WARNING
This product can expose you to chemicals including formaldehyde, which is known to the State of California to cause cancer, and methanol, which is known to the State of California to cause birth defects or other reproductive harm. For more information go to www.P65Warnings.ca.gov.

Potential Hazard	Label	Description
Class 4 Laser		The instrument is a Class 1 laser product that contains a Class 4 laser. See Laser Safety on page 60 .
Heat hazard		The nest has a hot surface and exposure can cause burns.

Laser Safety

The AVITI24 System is certified as a Class 1 laser product per the US Federal Product Performance Standard for Laser Products requirements described in 21 CFR Subchapter J. The exception to these requirements is the deviations described in FDA Laser Notice #56. The product is classified per IEC/EN 60825-1:2014.

WARNING

Adjusting or performing procedures other than those described in this guide or other Element guides can result in hazardous radiation exposure.

Class 4 Laser

The instrument is a Class 1 laser product that contains a Class 4 laser. The Class 4 laser produces Class 4 levels of visible laser radiation, which can be hazardous to eyes and skin. Protective shells and safety interlocks prevent exposure or access to laser radiation levels that exceed Class 1 during operation, maintenance, or normal service.

The following figure depicts the label that identifies noninterlocked portions of the shells that prevent access to laser radiation. Additionally, the nest bay and both reagent bays contain barcode scanners that emit Class 1 levels of laser radiation.

Label identifying noninterlocked locations



Operating Conditions

Do not operate an AVITI24 System with bypassed interlocks, damaged shells, or any portion of the shells removed. These conditions make Class 4 levels of laser radiation possible and risk exposure to direct or reflected laser light.

Only Element service personnel, Element-authorized agents, or Element-trained personnel can perform services that require internal interlock bypass or removal of portions of the shells. If you are present during service, take the proper safety precautions to mitigate the risk of direct and reflected laser light.

Product Compliance

The AVITI24 System meets the Canadian, EU, South Korean, UK, and US requirements for safety and electromagnetic compatibility (EMC). The system has been tested to and complies with the standards in the following sections.

US and Canadian Safety and EMC Standards

The AVITI24 System is certified to the following safety standards:

- IEC 60825-1, safety of laser products
- IEC 61010-1, general safety requirements for electrical equipment for measurement, control, and laboratory use
- IEC 61010-2-010, particular requirements for laboratory equipment for the heating of materials
- IEC 61010-2-081, particular requirements for automatic and semiautomatic laboratory equipment for analysis and other purposes

The system also has been tested to and complies with the following EMC requirements:

- FCC 47 CFR Part 15, title 47: telecommunication; part 15 – radio frequency (RF) devices
- ICES-003, information technology equipment (including digital apparatus)

FCC Compliance Statement

This device complies with part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

EU Safety and EMC Standards

The AVITI24 System has been tested to and complies with the following safety standards:

- Low Voltage Directive 2014/35/EU
 - » EN 61010-1, general safety requirements for electrical equipment for measurement, control and laboratory use
 - » EN 61010-2-010, particular requirements for laboratory equipment for the heating of materials
 - » EN 61010-2-081, particular requirements for automatic and semiautomatic laboratory equipment for analysis and other purposes
 - » EN 60825-1, safety of laser products

The system has been tested to and complies with the following EMC standards:

- EMC Directive 2014/30/EU, EMC requirements
 - » EN 61326-1, general EMC requirements for electrical equipment for measurement, control and laboratory use

The system also complies with the Restriction of Hazardous Substances (RoHS) Directive (2011/65/EU) as amended by the Directive (EU) 2015/863. The directives restrict the use of certain hazardous substances in electrical and electronic equipment.

UK Safety and EMC Standards

The AVITI24 System has been tested to and complies with the following safety standards:

- S.I. 2016 No. 1101, general safety regulations

- » BS EN 61010-1, general safety requirements for electrical equipment for measurement, control and laboratory use
- » BS EN IEC 61010-2-010, particular requirements for laboratory equipment for the heating of materials
- » BS EN IEC 61010-2-081, particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes
- » BS EN 60825-1, safety of laser products

The system also has been tested to and complies with the following EMC standards:

- S.I. 2016 No.1091, EMC requirements
 - » BS EN IEC 61326-1, general EMC requirements for electrical equipment for measurement, control and laboratory use

South Korea EMC and Regulatory Compliance

The AVITI24 System has been tested to and complies with the following EMC standards:

- KS C 9610-6-2, electromagnetic immunity standards for equipment used in industrial environments
- KS C 9610-6-4, electromagnetic emission standards in industrial environments

Additional regulatory information to comply with South Korean regulations (in Korean and English):

- 이 기기는 업무용 환경에서 사용할 목적으로 적합성평가를 받은 기기로서 가정용 환경에서 사용하는 경우 전파간섭의 우려가 있습니다.
- "This equipment has been evaluated for its suitability for use in a business environment. When used in a residential environment, there is a concern of radio interference."

Regulatory Markings

The following markings indicate that the instrument complies with conformity requirements, including EMC and safety requirements, for Australia, Canada, the EU, South Korea, the UK, and the US.

Symbol	Description
	Nemko Electrical Safety Certification Mark for US and Canada
	Australia Regulatory Compliance Mark
	European Conformity (CE) Marking
	UK Conformity Assessed Marking
 R-R-EB6-710-00975	South Korea Conformity Assessment Marking

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Document History

Revision	Description of Change
April 2025 Document # MA-00051 Rev. D	<ul style="list-style-type: none"> • Updated the General tab description in Settings. • Updated note in Run Monitoring to include content for cytoprofilng. • Added links to sequencing and cytoprofilng metrics and charts. • Updated the maintenance wash requirement. • Added ElemBio Catalyst to Supported Storage Connections. • Added ElemBio Catalyst to Add-Ons. • Added Polony Density to Add-Ons. • Updated the estimated completion time for software updates. • Added recommendation to perform software updates during downtime. • Updated title of Perform a Remote Update to Perform an Over-the-Air update and added power cycle steps. • Updated USB drive specifications for manual software updates. • Added power cycle steps for manual software updates. • Added replacement intervals for air filters. • Updated Nemko symbol in Regulatory Markings.
January 2025 Document # MA-00051 Rev. C	<ul style="list-style-type: none"> • Removed description of cartridge shipping configurations, such as shipping locks or shipping cover.
December 2024 Document # MA-00051 Rev. B	<ul style="list-style-type: none"> • Added list of additional documentation for workflow instructions. • Added Trinity to the list of kits that are not compatible with individually addressable lanes. • Specified that Cloudbreak cartridges include shipping locks and Trinity cartridges include a thermoform cover. • Updated images of Teton flow cell aligner and sealer to the latest configuration. • Removed the lid from wash tray description.
October 2024 Document # MA-00051 Rev. A	<ul style="list-style-type: none"> • Initial release

Technical Support

Visit the [Documentation page](#) on the Element Biosciences website for additional guides and the most recent version of this guide. For technical assistance, contact Element Technical Support.

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ELEMENT BIOSCIENCES

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EXHIBIT 23



Article

<https://doi.org/10.1038/s41587-023-01750-7>

Sequencing by avidity enables high accuracy with low reagent consumption

Received: 15 August 2022

Accepted: 15 March 2023

Published online: 25 May 2023



A list of authors and their affiliations appears at the end of the paper

We present avidity sequencing, a sequencing chemistry that separately optimizes the processes of stepping along a DNA template and that of identifying each nucleotide within the template. Nucleotide identification uses multivalent nucleotide ligands on dye-labeled cores to form polymerase–polymer–nucleotide complexes bound to clonal copies of DNA targets. These polymer–nucleotide substrates, termed avidites, decrease the required concentration of reporting nucleotides from micromolar to nanomolar and yield negligible dissociation rates. Avidity sequencing achieves high accuracy, with 96.2% and 85.4% of base calls having an average of one error per 1,000 and 10,000 base pairs, respectively. We show that the average error rate of avidity sequencing remained stable following a long homopolymer.

Avidity sequencing chemistry enables a diversity of applications that include single-cell RNA sequencing (RNA-seq) and whole-human-genome sequencing. For the human sample HG002, avidity sequencing reached a single-nucleotide polymorphism (SNP) F1 score of 0.9958 and small-indel F1 score of 0.9954.

Over the past 15 years, highly parallel sequencing methods have enabled a broad set of applications^{1–8}. Multiple technologies have been introduced during this time, each having various strengths and limitations⁹. The technologies vary by accuracy, read length, run time and cost. The most widely used method uses highly parallel and accurate short-read sequencing, described in ref. 10 and termed sequencing by synthesis (SBS).

The SBS methodology sequences DNA by controlled (that is, one at a time) incorporation of modified nucleotides¹¹. The modifications consist of a 3' blocking group and a dye label^{12,13}. The blocking group ensures that only a single nucleotide is incorporated, and the dye label enables identification of each nucleotide following an imaging step. The blocking group and label are subsequently removed, completing the sequencing cycle. The cycle is repeated with the incorporation of the next blocked and labeled nucleotide. Incorporation of the modified nucleotide meets two objectives: to advance the polymerase along the DNA template and to differentially label the incorporated nucleotide for base identification. Although combination of the two processes is efficient, it prevents independent optimization of the processes. High-yielding and rapid incorporation requires micromolar concentrations of nucleotides to drive the polymerizing reaction^{14–18}. The alternative, of allowing longer incorporation times, results in longer cycle times that have an additive effect over 300 cycles of stepwise sequencing.

We present a different sequencing chemistry, termed avidity sequencing, that separates and independently optimizes the controlled incorporation and nucleotide identification steps to achieve increased base-calling accuracy relative to SBS while reducing the concentration of key reagents to nanomolar scale. To advance this approach, we first had to overcome the technical challenge of signal persistence. For example, a potential strategy for separation of the steps described above could be to first incorporate a 3' blocked but unlabeled nucleotide and then to bind a complementary labeled nucleotide to the subsequent base in the template for base identification. This approach is problematic because the dissociation rate for single nucleotides from a polymerase–template complex is large, and the polymerase–nucleotide complex does not remain stable throughout imaging unless prohibitively high concentrations of nucleotides are present in the bulk solution. To overcome this challenge, we used avidity.

Avidity refers to the accumulated strength of multiple affinities of individual noncovalent binding interactions, which can be achieved when multivalent ligands tethered in close proximity simultaneously bind to their targets¹⁹. Coincident binding increases ligand affinity and residence time²⁰. As an example of the potential impact of avidity on both affinity and decreased dissociation rate, Zhang et al.²¹ demonstrated that, by changing a monomeric to a pentameric nanobody, it is possible to decrease dissociation rates by three to four orders of magnitude. Our approach was to use avidity for nucleotide detection within the sequencing chemistry (Fig. 1). We demonstrate here that avidity sequencing achieves accuracy, surpassing an average of one error per 10,000 base pairs (bp) (Q40), and enables a diversity of applications that include single-cell RNA-seq and whole-human-genome

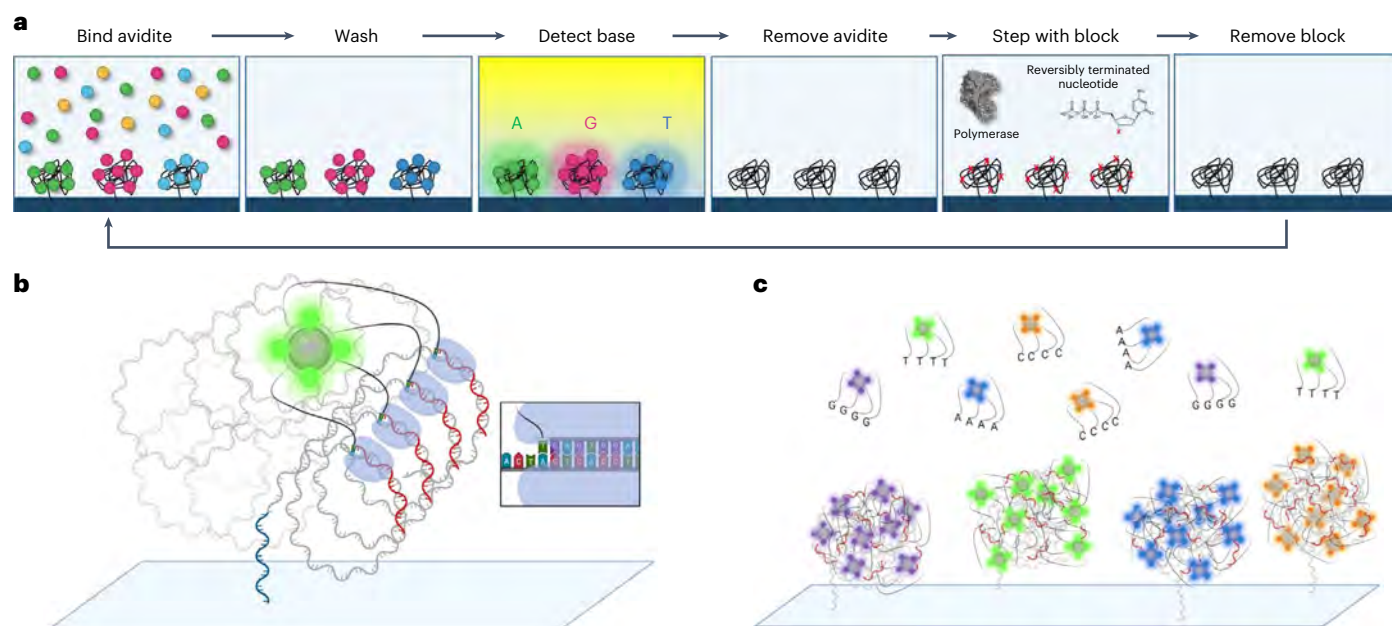


Fig. 1 | Avidity sequencing workflow and scheme. a, Sequencing by avidity. A reagent containing multivalent avidite substrates and an engineered polymerase are combined with DNA polonies inside a flowcell. The engineered polymerase binds to the free 3' ends of the primer-template of a polony and selects the correct cognate avidite via base-pairing discrimination. The multivalent avidite interacts with multiple polymerases on one polony to create avidity binding that reduces the effective K_d of the avidite substrates 100-fold compared with a monovalent dye-labeled nucleotide, allowing productive binding of nanomolar concentrations. Multiple polymerase-mediated binding events per avidite ensure a long signal persistence time. Imaging of fluorescent, bound avidites enables base classification. Following detection, avidites are removed from the polonies. Extension by one base using an engineered polymerase incorporates an unlabeled, blocked nucleotide. A terminal 3' hydroxyl is regenerated on the DNA

strand, allowing repetition of the cycle. **b**, Rendering of a single avidite bound to a DNA polony via polymerase-mediated selection. The initial surface primer used for library hybridization and extension during polony formation is shown in blue. Sequencing primers (red) are shown annealed to the single-strand DNA polony (gray). Each arm of the avidite (black) connects the avidite core containing multiple fluorophores (green) to a nucleotide substrate. The polymerase bound to the sequencing primer selects the correct nucleotide to base pair with the templating base (inset). The result is multiple base-mediated anchor points noncovalently attaching the avidite to the DNA polony. **c**, Rendering of multiple DNA polonies with template-specific avidites bound during the binding step of the cycle (polymerase not shown for simplicity). Many avidites bind to each DNA polony generating a fluorescent signal during detection. Multiple long, flexible polymer linkers connect the core to the nucleotide substrates.

sequencing. We also demonstrate an improved ability of this chemistry to sequence through homopolymer sequences.

Results

Before sequencing, DNA fragments of interest were circularized and captured on the surface of a flowcell. Clonal copies of DNA fragments were then created through rolling circle amplification, generating approximately 1 billion concatemers on the flowcell surface^{22–25}. The resulting concatemers, referred to as polonies using the original term coined by Church and collaborators²⁶, were used as the DNA substrate for sequencing. In contrast to the DNA nanoballs developed by Complete Genomics, polonies are amplified on-instrument following library hybridization to the flowcell²⁷. This approach simplifies user workflow and eliminates the possibility that DNA fragments may interact in solution during the amplification process. We then constructed the avidite: a dye-labeled polymer with multiple, identical nucleotides attached. In the presence of a polymerase, the avidite was able to bind multiple complementary nucleotides specifically in concatemer copies of a DNA fragment within a polony. A polymerase and a mixture of four avidites, each corresponding to a particular label and nucleotide, were applied to the flowcell and used for base discrimination. The avidite was not incorporated, but provided a stable complex while enabling removal under specifically formulated wash conditions. Removal of the avidite left no modifications in the synthesized strand. The avidites decreased the required concentration of reporting nucleotides by 100-fold relative to single-nucleotide binding, yielded negligible dissociation rates and obviated the need to have nucleotides present in the bulk solution. A low avidite concentration leads to reduced use of fluorophores relative to the strategy of using

high-concentrations of dye-labeled nucleotides. The advent of the avidite enabled us to separate the process of stepping along the DNA template from the process of identifying each nucleotide, and to optimize each for quality and reagent consumption. Figure 1a shows a complete cycle of avidity sequencing, Fig. 1b depicts a single avidite interacting with multiple DNA copies within a polony and Fig. 1c shows many avidites specifically bound to several polonies on the surface. Additional detail on the structure of one version of an avidite is provided in Extended Data Fig. 1.

Avidity sequencing overcomes the kinetic challenges of generating a signal by incorporation of a dye-labeled monovalent nucleotide. In bulk solution, incorporation of a dye-labeled nucleotide is limited by a specificity constant (k_{cat}/K_m) that governs the observed rate of productive nucleotide binding and incorporation²⁸. A specificity constant of $0.54 \pm 0.22 \mu\text{M}^{-1} \text{s}^{-1}$ for monovalent dye-labeled nucleotides using an engineered polymerase was observed resulting from a maximum rate of incorporation (k_{pol}) of $0.86 \pm 0.14 \text{s}^{-1}$ and an apparent dissociation constant K_d ($K_{d,app}$) of $1.6 \pm 0.6 \mu\text{M}$ (Fig. 2a). This apparent K_d reflects the K_m of a kinetic system not in equilibrium rather than the true K_d of the nucleotide substrate²⁹. To achieve complete product turnover, this high apparent K_d can be overcome either by using increased concentrations of fluorescent nucleotide substrate or allowing longer incorporation time for completion of the reaction. Both paths used to overcome this substrate limitation have the undesirable consequence of either high cost or long cycle time. Together, the use of avidity substrates and DNA polonies containing many copies of substrate DNA in close proximity overcomes the limitations of incorporating a monovalent dye-labeled nucleotide.

Using binding of the four labeled avidites for base identification established a binding equilibrium that reached saturation based on

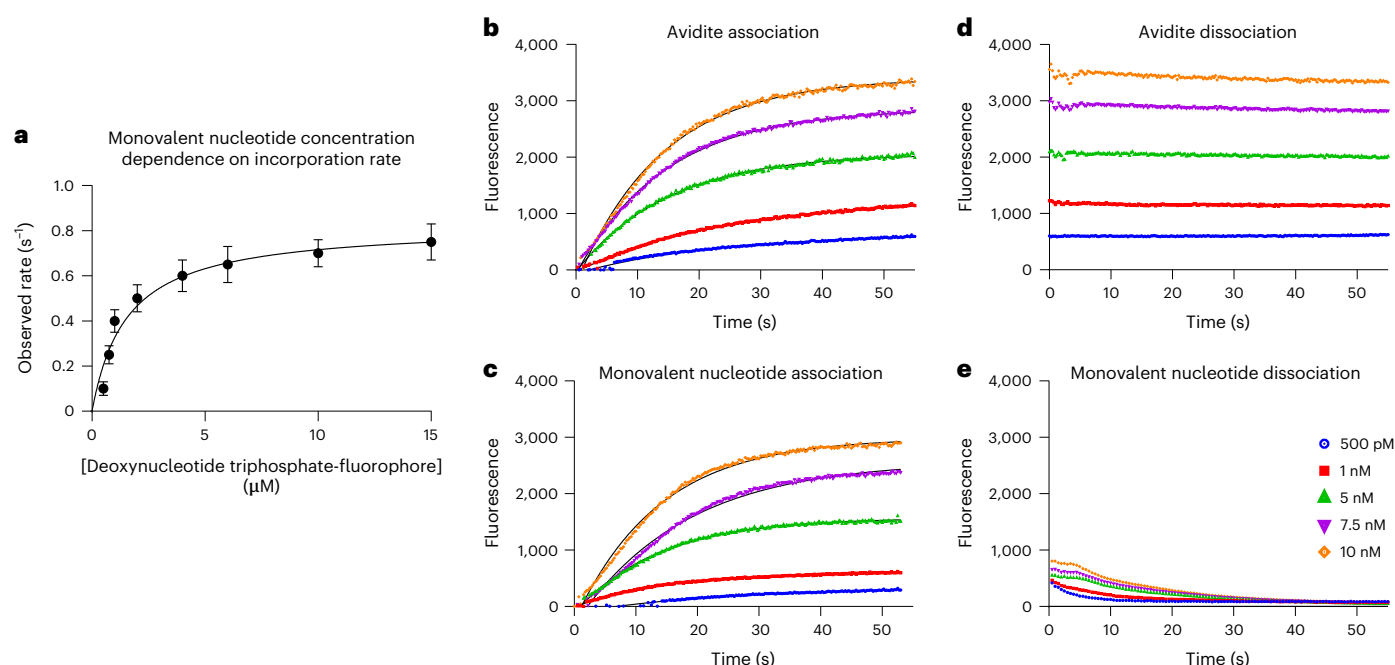


Fig. 2 | Nucleotide and avidite binding kinetics. **a**, Monovalent fluorophore-labeled nucleotide concentration dependence of the observed rate of incorporation. Time series were performed at each concentration and fit to a single exponential equation to derive a rate. Observed rates were plotted as a function of concentration and fit to a hyperbolic equation, deriving a value of

$k_{\text{pol}} = 0.86 \pm 0.14 \text{ s}^{-1}$ and $K_{\text{d,app}} = 1.6 \pm 0.6 \mu\text{M}$. **b,c**, Real-time association kinetics of signal generation resulting from reacting multivalent avidite substrates (**b**) and monovalent nucleotides (**c**) with DNA polonies. **d,e**, Real-time measurement of signal decay following flow cell washing for imaging of multivalent avidite substrates (**d**) and monovalent nucleotides (**e**).

substrate concentration within 30 s to generate signal, rather than relying on catalysis. The binding kinetics of this interaction were monitored using real-time data collection to observe avidites binding to polonies with an association rate ($k_{\text{on,avidite}}$) of $271 \pm 82 \text{ nM}^{-1} \text{ s}^{-1}$ (Fig. 2b). This observed association occurred within the limit of error of a single fluorescently labeled monovalent nucleotide (Fig. 2c). Major differences were observed in the dissociation kinetics of avidite substrates versus monovalent nucleotides. Avidite substrates bound to the DNA polonies tightly with no measurable dissociation over the >1-min timescale needed for imaging and base calling (Fig. 2d). This is in sharp contrast to fluorescently labeled monovalent nucleotides, which dissociated rapidly during the wash step following binding and then continued to dissociate during imaging (Fig. 2e). The negligible dissociation rate resulted in decreased K_{d} of more than two orders of magnitude for avidites compared with monovalent nucleotides. With near-zero avidite dissociation rates, a persistent signal was achieved without the presence of free avidites in bulk solution, eliminating background. Without avidity, dissociation kinetics with monovalent nucleotides showed a fourfold signal decrease at the beginning of imaging due to rapid dissociation, as a result of disruption of the binding equilibrium during reagent exchange (Fig. 2e).

Sequencing instrumentation

Avidity sequencing was performed on the AVITI commercial sequencing system. Briefly, the instrument is a four-color optical system with two excitation lines of approximately 532 and 635 nm. The four-color system is created using an objective lens, multiple tube lenses and multiple cameras for simultaneous imaging of four spectrally separated colors. The detection channels for emission are centered at approximately 553, 596, 668 and 716 nm, respectively. Reagents are delivered using a selector valve and syringe pump to perform reagent cycling. The instrument contains two fluidics modules and a shared imaging module, enabling parallel utilization of two flowcells. Subsequent to image collection, data were streamed through an onboard processing

unit that performs image registration, intensity extraction and correction, base calling and quality score assignment (Methods).

Accuracy of avidity sequencing

To evaluate the accuracy of avidity sequencing, 20 sequencing runs were performed using a well-characterized human genome. Sequencing data were used to train quality tables according to the methods of Ewing et al.³⁰, but with modified predictors. Quality tables were then applied to independent sequencing runs. Figure 3 shows the data quality obtained in a representative run not used for training. Quality scores were well calibrated across the entire range, meaning that predicted quality matched observed quality as determined by alignment to a known reference. Combined over reads 1 and 2, 96.2% of base calls were >Q30 (an average of one error per 1,000 bp) and 85.4% >Q40, with a maximum of Q44, or approximately one error in 25,000 bases. For comparison, a publicly available PCR-free NextSeq 2000 dataset was downloaded from the Illumina public demo set repository (<https://basespace.illumina.com/datacentral>) and a publicly available NovaSeq 6000 dataset (<https://console.cloud.google.com/storage/browser/brain-genomics-public/research/sequencing/fastq>). The NextSeq 2000 and NovaSeq 6000 datasets had 90.1% and 92.7% of data >Q30, respectively, and none of the base calls exceeded Q40.

To obtain an additional measure of accuracy, we used the same datasets to compute the percentage of k -mers ($k = 1, 2, 3$) containing at least one mismatch after alignment to a well-characterized reference. Known SNP sites were masked before the comparison. When compared with NextSeq 2000 and NovaSeq 6000, we found that AVITI had the highest accuracy across four out of four 1-mers, 16 out of 16 2-mers and 58 out of 64 3-mers (Extended Data Fig. 2).

Homopolymer sequencing

Sequencing through long homopolymers has posed challenges for multiple sequencing technologies^{31,32}. Although SBS improves homopolymer sequencing relative to flow-based technologies,

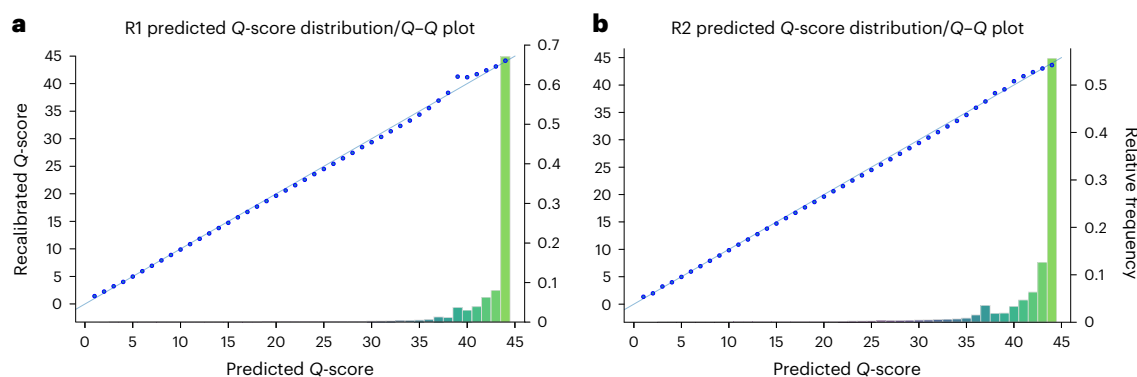


Fig. 3 | Predicted and observed quality scores for a 2×150 -bp sequencing run of human genome HG002. a, Read 1 (R1). b, Read 2 (R2). Points on the diagonal indicate that predicted scores match observed scores. The histograms show that the majority of the data points are $>Q40$.

the error rates of reads that pass through long homopolymer regions increase substantially³³. Correction algorithms have been proposed to circumvent the inherent challenges with base-calling post-homopolymer repeats³⁴, but the exact cause has not been fully established in the literature. In contrast to SBS, avidity sequencing leverages rolling circle amplification, polymerases evolved to accommodate the avidite complex formation and a separate polymerase evolved for efficient incorporation of unlabeled and 3' blocked nucleotides. We evaluated the impact of these differences on sequencing through long homopolymers. Specifically, homopolymers of length 12 or more nucleotides were used to assess the accuracy of reads before and after homopolymer regions. Figure 4 shows the results comparing avidity sequencing with SBS, averaged across the ~700,000 homopolymer loci of length 12 or more. Average error rate of avidity sequencing remained stable following a long homopolymer (controlling for the fact that post-homopolymer stretch occurs in later cycles of a read). By contrast, the error rate of SBS reads increased by more than a factor of five following homopolymer stretches. Extended Data Fig. 3 shows the histogram of pairwise error rate differences between avidity sequencing and SBS for all long homopolymer loci. The avidity sequencing error rate outperformed SBS in $>97\%$ of cases and the magnitude of difference is correlated with homopolymer length (Fig. 5). Extended Data Fig. 4 shows representative loci from the 95th, 50th and fifth percentiles of the histogram.

Single-cell RNA-seq

To demonstrate sequencing performance across common applications, single-cell RNA expression libraries were prepared and sequenced. Two libraries from a reference standard consisting of human peripheral blood mononuclear cells were generated using the 10X Chromium instrument. The two libraries contain RNA from roughly 10,000 and 1,000 cells, respectively. Following circularization, the libraries were sequenced to generate paired-end reads with read lengths of 28 and 90 for reads 1 and 2, respectively, as recommended by the vendor. The analysis was done using Cell Ranger (<https://support.10xgenomics.com/single-cell-gene-expression/software/pipelines/latest/installation>). Because this reference standard is used by 10X Genomics to evaluate sequencing performance, a set of metrics and guidelines to assess sequencing results is provided along with the biological material. Extended Data Table 1 shows each metric, the guideline values from 10X Genomics and the performance of each sequenced library. All metrics were within the guide ranges, and metrics pertaining to sequencing quality exceeded the thresholds provided.

Whole-human-genome sequencing

Another common application is human-whole-genome sequencing. This application challenges sequencer accuracy to a greater extent than measurement of gene expression because the latter requires only

accurate alignment while the former depends on nucleotide accuracy to resolve variant calls. To demonstrate performance for this application, the well-characterized human sample HG002 was prepared for sequencing using a Covaris shearing and PCR-free library preparation method and sequenced with 2×150 -bp reads. The run generated 1.02 billion passing filter paired-end reads with a duplicate rate of 0.58% (0.11% classified as optical duplicates by Picard (<https://broadinstitute.github.io/picard/>)). To underscore the impact of low duplicates, we compared the number of input reads with genomic coverage (Extended Data Fig. 5).

A FASTQ file with the base calls and quality scores was downsampled to 35-fold coverage and used as an input into the DNAScope analysis pipeline from Sentieon. SNP and indel calls achieved F1 scores of 0.995 and 0.996, respectively. Extended Data Table 2 shows variant-calling performance for SNPs and small indels on the GIAB-HC regions. Sensitivity, precision and F1 scores are shown. The performance on SNPs and indels is comparable. Extended Data Fig. 6 shows the F1 score for SNPs and indels across all GiAB stratifications with at least 100 variants in the truth set.

Extensibility of avidity sequencing

To assess the extensibility of avidity chemistry we continued a sequencing run beyond 150 bp to generate a 1×300 dataset from an *Escherichia coli* library. To achieve this we used both an optimized polymerase and an optimized reagent formulation. Figure 6a shows quality scores as a function of sequencing cycle. Because quality scores were not trained to these lengths, the scores are approximate. Figure 6b shows the *E. coli* error rate as a function of cycle number based on alignment to the known reference strain. The error rate of the final cycle was 1.9% and that at cycle 150 was 0.1%. Error calculations were based on the vast majority of the data with a pass filter rate for the run of $>99.6\%$ and Burrows–Wheeler aligner (BWA) settings aimed at strongly discouraging soft clipping (no cycles with soft clipping $>0.04\%$). The enzymes and formulations developed for this run will be leveraged as we continue to identify extensions and improvements.

Discussion

We present a sequencing chemistry that achieves improved quality and lower reagent consumption by independent optimization of nucleotide incorporation and signal generation. Although other chemistries have proposed the separation of incorporation and signal generation³⁵, the avidite concept benefits from the fact that multiple nucleotides on the avidite bind multiple copies of the DNA template within a polony, which decreases dissociation rate constant and the labeled reagent concentration requirement for base classification. Furthermore, the avidite construct is modular. The core can be swapped for a different substrate. Both number and type of dye molecules are configurable, and many

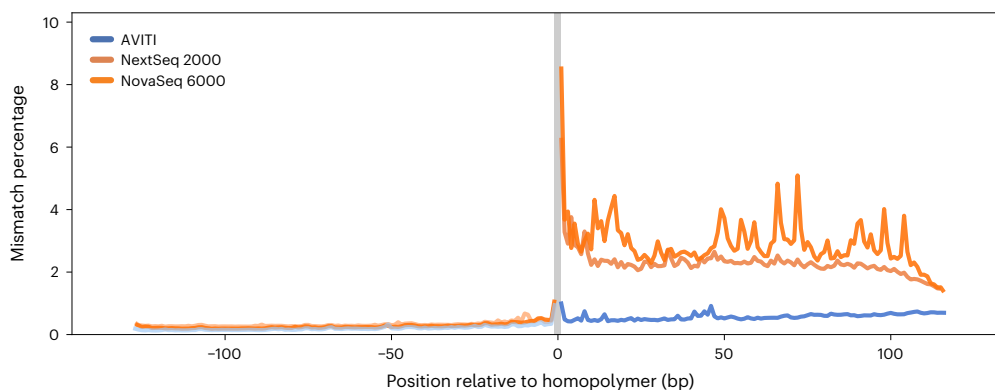


Fig. 4 | Post-homopolymer performance across platforms. Mismatch percentages of AVITI, NovaSeq 6000 and NextSeq 2000 reads before and after homopolymers of length 12 or greater.

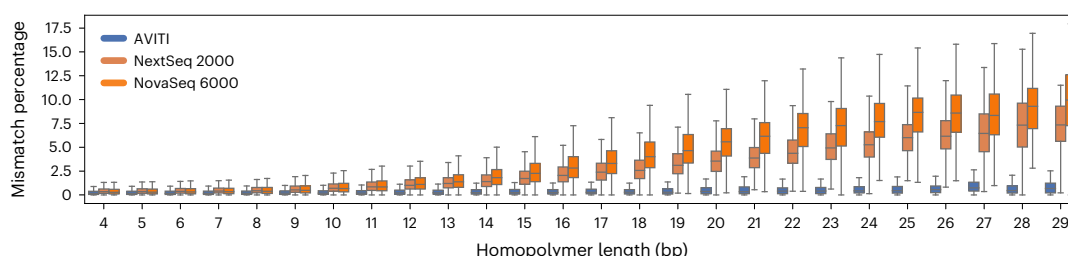


Fig. 5 | Comparison of mismatch rate following homopolymers of length between four and 29. Mismatch percentage difference between avidity sequencing and SBS increases with homopolymer length. The box plot shows median, quartiles and whiskers, which are 1.5× interquartile range.

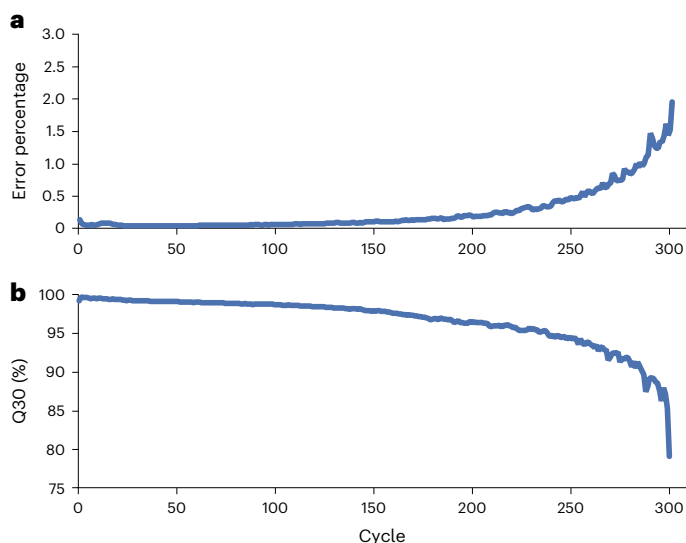


Fig. 6 | Performance of a 300-cycle *E. coli* sequencing run. **a**, Percentage Q30 by cycle. Overall Q30 percentage exceeds 96% and end of read has 85% Q30. **b**, *E. coli* error rate as a function of cycle. Alignment settings strongly discourage soft clipping, and >99% of reads pass filter. Final cycle error rate was 0.019.

types of linkers can be used. The changes are straightforward to implement and do not require modification of the polymerase responsible for binding the nucleotides attached to the linkers. The modular design speeds technology improvement because each component can be optimized in parallel for increased signal, decreased cycle time, lower reagent concentration or any other potential axis of improvement.

The avidity chemistry described above has been implemented as part of a benchtop sequencing solution. The accuracy of the sequencer was demonstrated by training a quality model on human sequencing

data, which shows that in the majority of bases in an independent human-whole-genome sequencing run is >Q40. The high level of accuracy probably results from (1) the use of an engineered high-fidelity polymerase, (2) synergistic binding of multiple nucleotides on a single avidite to ensure only the correct cognate avidite binds to the polony and (3) a binding disadvantage for out-of-phase DNA copies within a polony that lack other out-of-phase neighbors to serve as avidity substrates. Future work will be required to investigate the relative contribution of each mechanism proposed above. In addition to overall accuracy improvements, the chemistry retains good performance in reads containing long homopolymers. The sequencer can be used in a wide range of applications, as exemplified by results for single-cell RNA-seq and for whole-human-genome sequencing. In both cases, reference standards were sequenced so that the quality of result could be assessed. The single-cell data exceeded the quality metric guidelines provided by 10X Genomics (<https://www.10xgenomics.com/compatible-products?query=&page=1>). The human genome variant-calling results showed high sensitivity and precision for both SNPs and small indels³⁶. The two benchmarking studies were selected due to the availability of well-characterized samples and because they represent very different use cases. However, these are only examples and other applications have been demonstrated, including whole-genome sequencing for rare disease³⁷, low-pass sequencing with imputation³⁸ and single-cell sequencing of DNA and RNA³⁹. Although the current implementation of avidity-based sequencing already achieves high accuracy and broad applicability, there are many improvement directions being explored. In addition to the initial demonstration of longer reads shown here, further quality improvements, shorter cycle times and higher densities are under development.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions

and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41587-023-01750-7>.

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Methods

Solution measurements of nucleotide incorporation

Solution measurements of nucleotide kinetics were performed using commercially available dATP-Cy5 (Jena Bioscience, catalog no. NU-1611-CY5-S). DNA substrates for solution kinetic assays were prepared by annealing a 5'-FAM-labeled primer oligo (purchased from IDT) and high-performance liquid chromatography-purified (5'-CGAGCCGTCCAACCTACTCA-3') with a template oligo (5'-ACGACCATGTTGAGTAGTTGGACGGCTCG-3'). Annealing was performed with 10% excess template oligo in the annealing buffer using a PCR machine to heat oligos to 95 °C, followed by slow cooling to room temperature over 60 min. Solution kinetics were performed by mixing a preformed enzyme–DNA complex with fluorescent nucleotide and MgSO₄ using a RQF3 Rapid Quench Flow (KinTek Corp.). The enzyme used was an engineered variant of *Candidatus altiarthaeales* archaeon. The final reaction was conducted in 25 mM Tris pH 8.5, 40 mM NaCl and 10 mM ammonium chloride at 37 °C. Extension products were separated from unextended primer oligos by capillary electrophoresis using a 3500 Series Genetic Analyzer (ThermoFisher) to achieve single-base resolution. Products were quantified and fit to a single exponential equation. The observed rates as a function of nucleotide concentration were then fit to a hyperbolic equation to derive apparent K_d ($K_{d,app}$) and rate of polymerization (k_{pol}).

Avidite synthesis and construction

Initial research scale avidites were constructed by dissolving 5 mg of 10 kD 4-arm-PEG-SG (Laysan Bio, catalog no. 4arm-PEG-SG-10K-5g) in 100 µl of 95% organic solvent (for example, ethanol) and 5 mM MOPS pH 8.0 to make a 50 mg ml⁻¹ solution (5 mM), 19 µl of which was combined with 1.5 µl of 10 mM dATP-NH₂ (7-deaza-7-propargylamin-2'-deoxyadenosin-5'-triphosphate; Trilink, catalog no. N-2068) and 8.0 µl of 3.75 mM 2 kD Biotin-PEG-NH₂ (Laysan Bio, catalog no. Biotin-PEG-NH2-2K-1g) in 95% organic solvent (for example, ethanol) and 5 mM MOPS pH 8.0. After mixing, 5 mM 10 kD 4-arm-PEG-SG was added. The final composition was 0.50 mM dA-NH₂, 1.0 mM biotin-PEG-NH2 (2 kD), 0.25 mM 4-arm-PEG-NHS, 85.5% organic solvent (for example, ethanol) and 4.5 mM MOPS pH 8.0. Following 1,000-rpm incubation at 25 °C for 90 min, the reaction volume was adjusted to 100 µl by the addition of MOPS pH 8.0. Purification was performed using a Biorad Biospin P6 column pre-equilibrated in 10 mM MOPS pH 8.0. The purified dATP-PEG–biotin complex was mixed with Zymax Cy5 Streptavidin (Fisher Scientific, catalog no. 438316) in a 2.5:1 volumetric ratio and allowed to equilibrate for 30 min at room temperature.

Real-time measurement of avidite association and dissociation

Real-time measurement of avidite binding kinetics was performed using an Olympus IX83 microscope at 545 and 635 nm excitation (Lumencor Light Engine) set to an approximate power density of about 1 W cm⁻², with an Olympus objective (catalog no. UCPLFLN20XPH) and a Semrock BrightLine multiband laser filter set (catalog no. LF405/488/532/635) containing a matching quad band exciter, emitter and dichroic. Flow rates of 60 µl s⁻¹ were used for reagent exchanges. Circular PhiX libraries were introduced to AVITI flow cells, hybridized in 3× SSC buffer for 5 min at 50 °C and cooled to room temperature. Amplification reagents were introduced into the flow cell to perform rolling circle amplification and amplify genomic DNA. The instrument was paused following polony generation and priming and the flowcell moved to the microscope. Custom control software was written to control all peripheral hardware and synchronize data collection with flow of materials into the sample. Data collection (4 fps) was triggered by flow of the avidity mix and collected for 55 s. Polonies in the field were localized by a spot-finding algorithm, and background-corrected intensities were extracted versus time. Experiments were performed

at 0.5 pM, 1 nM, 7.5 nM and 10 nM avidite or monovalent dye-labeled nucleotide concentrations. Substrates at the respective concentrations were combined with 100 nM engineered enzyme variant of *C. altiarthaeales* archaeon in the avidity on rate assay buffer formulation (25 mM HEPES pH 8.8, 25 mM NaCl, 0.5 mM EDTA, 5 mM strontium acetate, 25 mM ascorbic acid and 0.2% Tween-20). Avidites and nucleotides were labeled with Alexa Fluor 647. Higher-concentration data collection was limited by the ability to detect polony intensity from free avidite intensity at elevated concentrations. Off-rate measurements were performed by binding avidites to flowcell polonies, followed by washing with avidity on rate assay buffer and triggering of data collection.

Genomic DNA and next-generation sequencing library preparation

Human DNA from cell line sample HG002 was obtained from the Coriell Institute. Linear next-generation sequencing library construction was performed using a KAPA HyperPrep library kit (Roche, catalog no. 07962363001) according to published protocols. Finished linear libraries were circularized using the Element Adept Compatibility kit (catalog no. 830-00003). Final circular libraries were quantified by quantitative PCR with the standard and primer set provided in the kit. Circular library DNA was denatured using sodium hydroxide and neutralized with excess Tris pH 7.0 before dilution. Denatured libraries were diluted to 8 pM in hybridization buffer before loading onto the sequencing cartridge.

Single-cell 3' gene expression library circularization

Single-cell RNA-seq libraries were prepared from two lots of peripheral blood mononuclear cell suspension (10,000 and 1,000 cells) using the Chromium Next GEM Single Cell 3' Kit v.3.1 (catalog no. 1000268). Each library was quantified and individually processed for sequencing using the Adept Library Compatibility Kit (catalog no. 830-00003). Processed libraries were pooled and sequenced with 28 cycles for read 1, 90 for read 2 and index reads.

Sequencing instrument and workflow

Sequencing results were obtained with commercialized formulations of avidites, enzymes and buffers. Element Bioscience's AVITI commercial system (catalog no. 88-00001) was used for all sequencing data. AVITI 2 × 150 kits were loaded on the instrument (catalog no. 86-00001). Primary analysis was performed onboard the AVITI sequencing instrument, and FASTQ files were subsequently analyzed using a secondary analysis pipeline from Sentieon.

Sequencing primary analysis

Four images were generated per field of view during each sequencing cycle, corresponding to the dyes used to label each avidite. An analysis pipeline was developed that uses the images as input to identify the polonies present on the flowcell and to assign to each polony a base call and quality score for each cycle, representing the accuracy of the underlying call. The analysis approach has steps similar to those described in ref. 25. Briefly, intensity is extracted for each polony in each color channel; intensities are then corrected for color cross-talk and phasing and normalized to make cross-channel comparisons. The highest normalized intensity value for each polony in each cycle determines the base call. In addition to assigning a base call, a quality score corresponding to call confidences is also assigned. The standard Q -score definition is utilized where the Q -value is defined as $Q = -10 \times \log_{10} p$, where p is the probability that the base call is an error. Q -score generation follows the approach of Ewing et al., with modified predictors²¹, and is encoded using the phred+33 ASCII scheme. The predictors used for quality score training are (1) maximum intensity per polony across color channels; (2) clarity of each polony (defined as $(A + 1)/(B + 1)$, where A is the highest intensity across color channels and B is the second highest); (3) the sum

of phasing and prephasing estimates; and (4) the median clarity value taken across the 10% of the lowest-intensity colonies. The sequence of base call assignments and quality scores across the cycles constitutes the output of the run. These data are represented in standard FASTQ format for compatibility with downstream tools.

Quality score assessment

To assess the accuracy of quality scores (Fig. 3), the FASTQ files were aligned with BWA to generate BAM files. GATK BaseRecalibrator was then applied to the BAM, specifying files of publicly available known sites to exclude human variant positions.

K-mer error analysis

The same run used to generate recalibrated quality scores was analyzed via custom script for all *k*-mers of size 1, 2 and 3. The computation is based on 1% of a 35X genome to ensure adequate sampling of each *k*-mer. For example, each 3-mer is sampled at least 850,000 times (average 6.7 million). This figure is based on a publicly available run from each platform. For the instances of each *k*-mer, the percentage mismatching a variant-masked reference was computed. The same script was applied to a publicly available NovaSeq dataset for HG002 and a publicly available NextSeq 2000 dataset for HG001 (Demo Data for HG002 were not available). We tabulated the number of *k*-mers in which the percentage incorrect was lowest for AVITI among the three platforms compared.

Homopolymer analysis

A BED file provided by National Institute of Standards and Technology (NIST) genome-stratifications v.3.0, containing 673,650 homopolymers of length >11, was used to define regions of interest for homopolymer analysis (GRCh38_SimpleRepeat_homopolymer_gt11_slop5). Reads overlapping these BED intervals (using samtools view -L and adjusting for slop5) were selected for accuracy analysis. Reads with any of the following flags set were discarded: secondary, supplementary, unmapped or reads with mapping quality of 0. Reads were oriented in the 5'→3' direction and split into three segments: preceding the homopolymer, overlapping it and following it. The mismatch rate for each read segment was computed, excluding N-calls, softclipped bases and indels. For example, if a 150-bp read (aligned on the forward strand) contained a homopolymer in positions 100–120, the first 99 cycles were used to compute the error rate before the homopolymer and the last 30 to compute error rate following the homopolymer. Reads were discarded if the sequence either preceding or following the homopolymer was <5 bp in length. All reads were then stacked into a matrix according to their positional offset relative to the homopolymer, and error rate per post-offset was computed.

Average error rate was computed for avidity sequencing runs and for publicly available data from multiple SBS instruments, for comparison. Differences in mismatch percentage, across all BED intervals, between AVITI and NovaSeq were plotted in a histogram and examples showing various percentiles within the distribution were chosen for display via Integrative Genomics Viewer.

Publicly available datasets for NovaSeq were obtained from the Google Brain Public Data repository on Google Cloud (<https://console.cloud.google.com/storage/browser/brain-genomics-public/research/sequencing/fastq>). Publicly available NextSeq 2000 data were obtained from Illumina Demo Data on BaseSpace (<https://basespace.illumina.com/datacentral>).

Single-cell gene expression data analysis

Following sequencing, Bases2Fastq software was used to generate FASTQ files for compatible upload into 10X Cloud and subsequent analysis with the 10X Genomics Cell Ranger analysis package. Data visualization of single-cell gene expression profiling was generated using 10X Genomics Loupe Browser.

Whole-genome sequencing analysis

A FASTQ file with base calls and quality scores was downsampled to 35× raw coverage (360,320,126 input reads) and used as an input into Sentieon BWA followed by Sentieon DNAScope⁴⁰. Following alignment and variant calling, variant calls were compared with the NIST genome in Bottle Truth Set v.4.2.1 via the hap.py comparison framework to derive total error counts and F1 scores⁴¹. The results are computed based on the 3,848,590 SNV and 982,234 indel passing variant calls made by DNAScope.

1 × 300 Data generation

An *E. coli* library was prepared using enzymatic shearing and PCR amplification. The library was then sequenced for 300 cycles using new enzymes for stepping along the DNA template and for avidite binding. The reagent formulation with increased enzyme and nucleotide concentrations during the stepping process was used to improve stepping performance. The contact times for avidite binding and exposure were both reduced without performance losses, to decrease cycle time over the 600 cycles of sequencing. The displays show only 299 cycles of data, because cycle 300 was used only for prephasing correction. To minimize soft clipping during alignment the following inputs were used in the call to BWA-MEM: -E 6,6 -L 1000000 -S.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The avidity sequencing datasets described in the paper are available for download via the AWS CLI in the public bucket s3://avidity-manuscript-data/, pending upload to the sequence read archive under BioProject PRJNA869673. Publicly available datasets for NovaSeq were obtained from the Google Brain Public Data repository on Google Cloud (<https://console.cloud.google.com/storage/browser/brain-genomics-public/research/sequencing/fastq>). Publicly available NextSeq 2000 data were obtained from Illumina Demo Data on BaseSpace (<https://basespace.illumina.com/datacentral>).

Code availability

Scripts used for analysis are available via GitHub (<https://github.com/Elombio/AvidityManuscript2023>).

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40. Freed, D. et al. The Sentieon Genomics Tools—a fast and accurate solution to variant calling from next-generation sequence data. Preprint at *bioRxiv* <https://doi.org/10.1101/115717> (2017).
41. Krusche, P. et al. Author correction: Best practices for benchmarking germline small-variant calls in human genomes. *Nat. Biotechnol.* **37**, 567 (2019).

Acknowledgements

We thank J. Puglisi and T. Ben-Yehzekel for valuable comments and discussion during the writing of the paper.

Author contributions

The author list is divided into three sections, each in alphabetical order. Authors in the first section made equal contributions to the critical elements of the technology and paper development. Authors in the second category made specific technology contributions described within the paper. Authors in the third group helped to develop some aspects of the underlying technology that culminated in the final product. M.H. and M.P. shared in the intellectual supervision of the work.

Competing interests

All authors are current or former employees of Element Biosciences.
All authors may hold stock options in the company.

Additional information

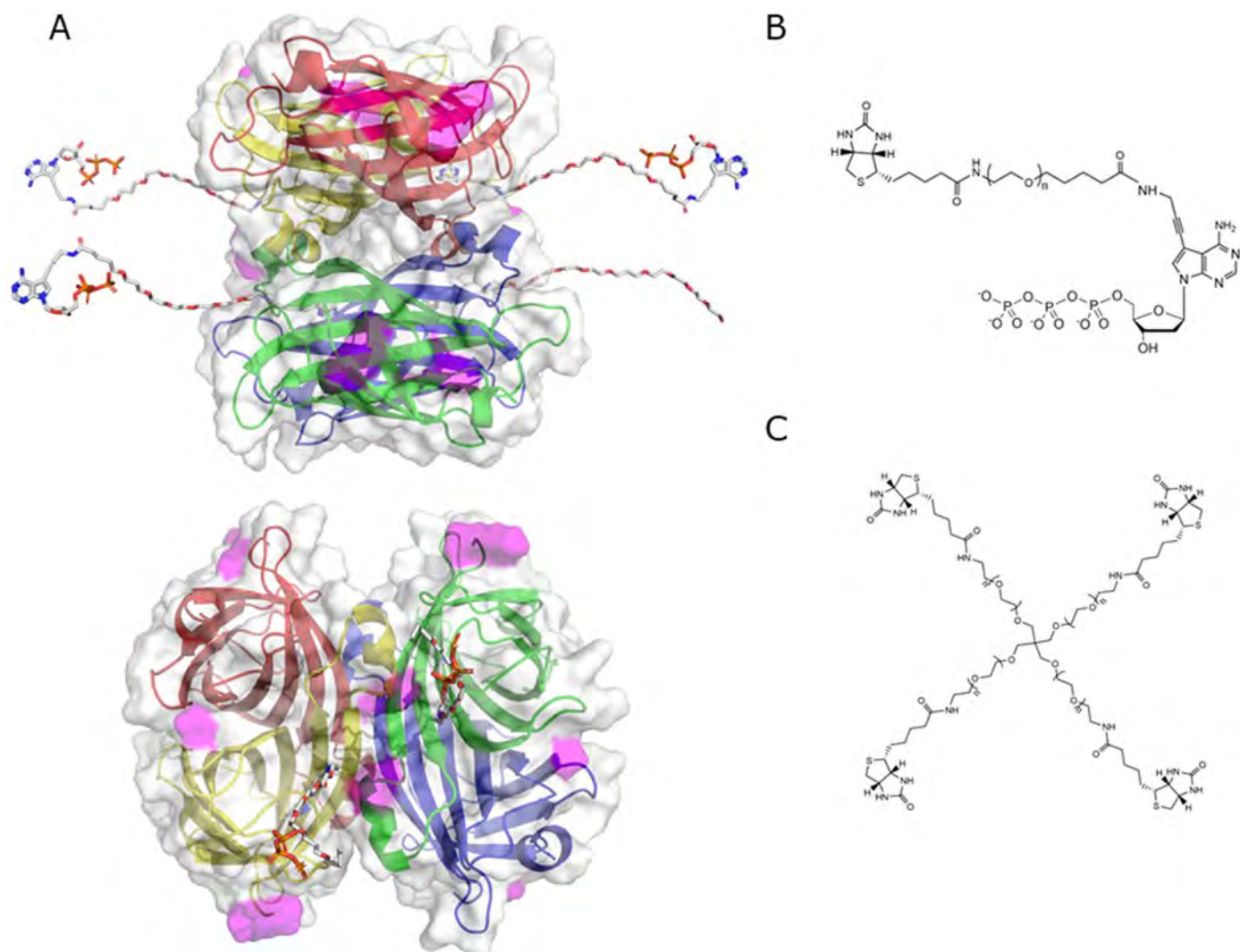
Extended data is available for this paper at
<https://doi.org/10.1038/s41587-023-01750-7>.

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41587-023-01750-7>.

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Peer review information *Nature Biotechnology* thanks Michael Quail, Kenneth Beckman, Nathanael Olson and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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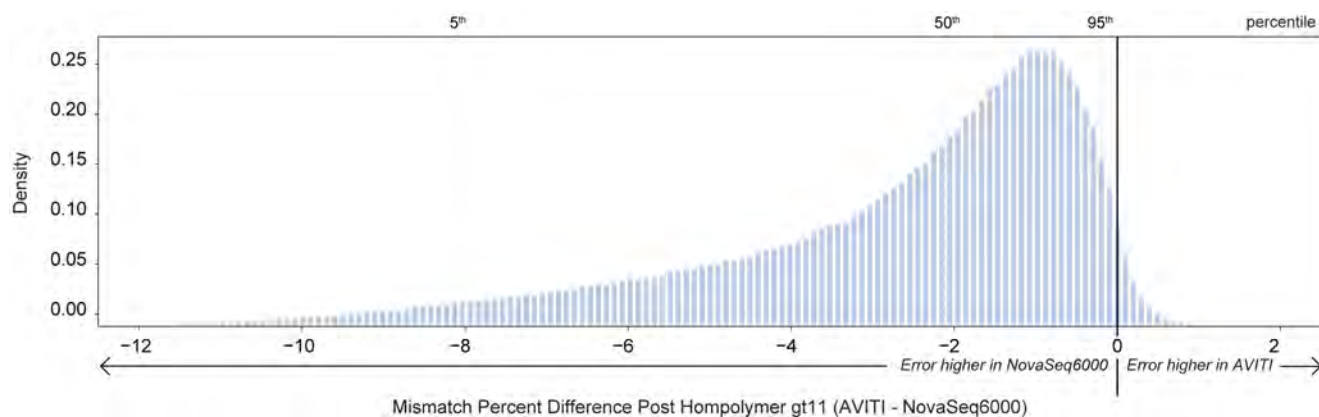


Extended Data Fig. 1 | Model of an avidite. (a) side and top views of a modeled avidite. The protein core consists of fluorophore labeled streptavidin. The monomers of tetrameric streptavidin are colored red, blue, green, and yellow. Dye conjugation sites through lysine-NHS chemistry are denoted in the surface rendering as magenta. Fluorophores are not pictured. Avidite arms are

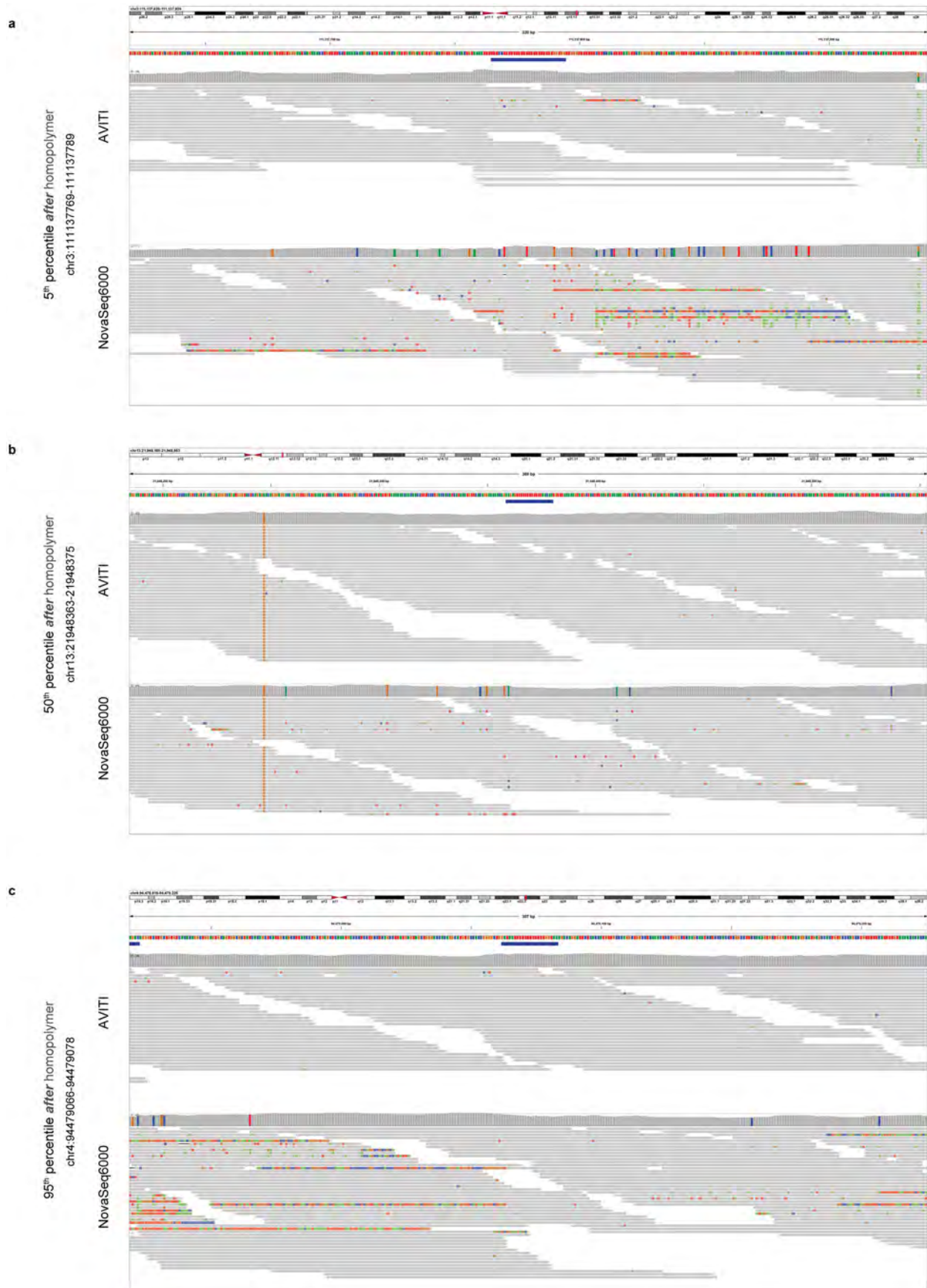
associated via a biotin interaction with the core streptavidin protein. Arms are mixed stoichiometrically to achieve averages of three nucleotide containing arms and one linker to additional cores. Molecules conjugated to have been shortened in this representation. (b) Structure of an avidite arm. (c) Structure of the 4-arm linker connecting avidite cores.



Extended Data Fig. 2 | Percentage of instances that a k-mer contained at least one mismatch compared across 3 instruments. Panels **a**, **b**, and **c** display 1-mers, 2-mers, and 3-mers, respectively. The bars are sorted by AVITI contexts from most to least accurate.

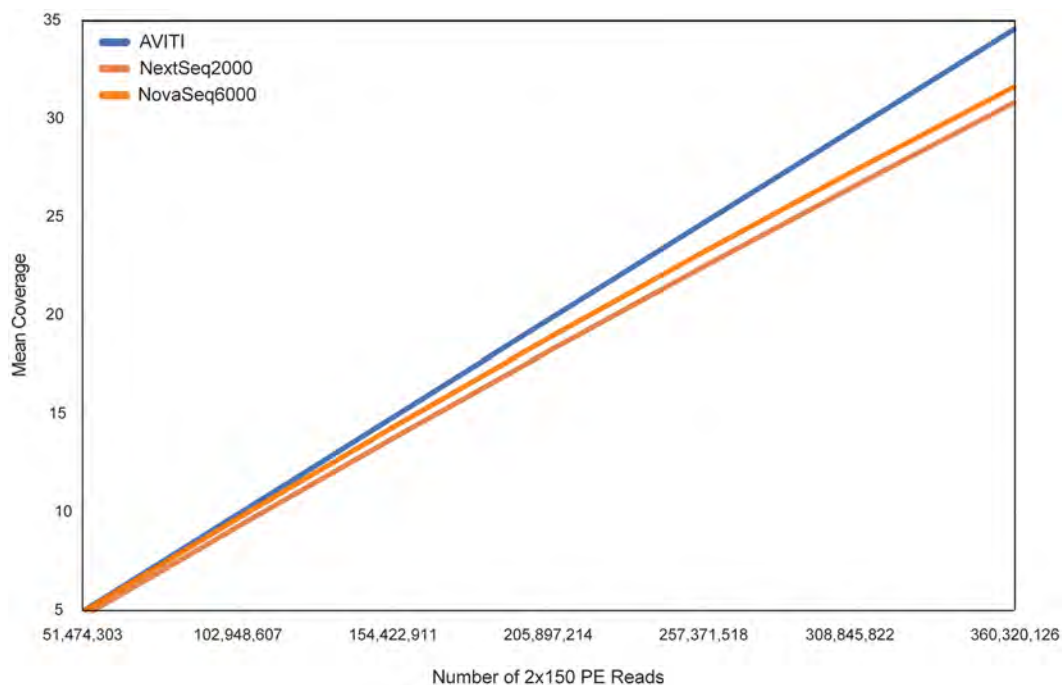


Extended Data Fig. 3 | Histogram of pairwise error differences. Difference was selected as the metric to cancel the effects of human variants from the mismatch percent.

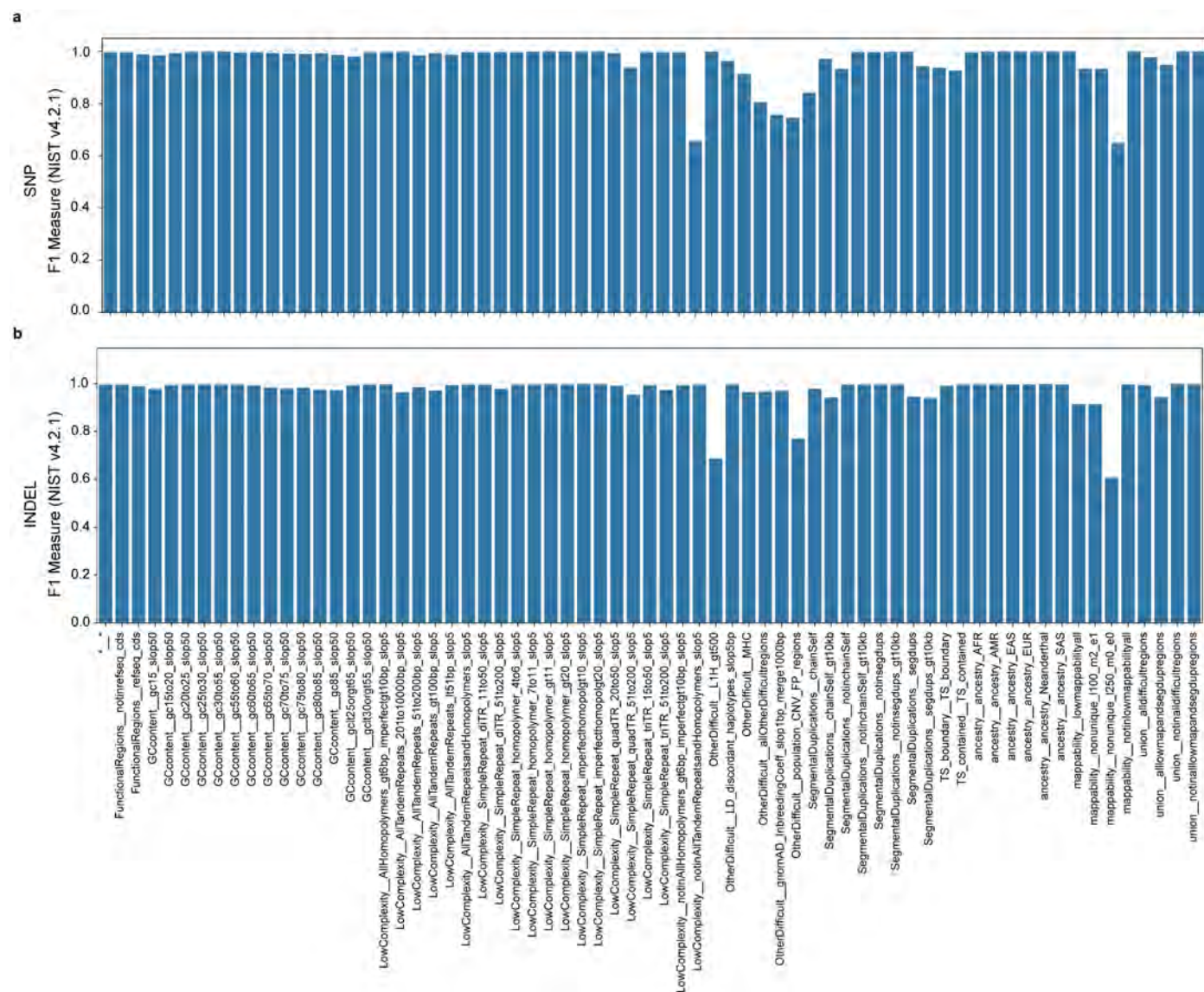


Extended Data Fig. 4 | IGV display of homopolymer loci at the 5th, 50th, and 95th percentile of AVITI minus NovaSeq mismatch percent (corresponding to the dashed lines of Extended Data Fig. 3). The red bar at the top indicates

the homopolymer. Colors within the IGV read stack correspond to mismatches and softclipping. Only mismatches contribute to the error rate calculation and softclipped bases are ignored.



Extended Data Fig. 5 | Comparison of read number vs genomic coverage computed via Picard for PCR-free whole genome data. AVITI most closely matches the 45-degree line due to the low duplicate rate.



Extended Data Fig. 6 | F1 Score of SNPs and indels across GiaB stratifications. F1 score for SNPs and indels stratified by all GiaB regions with at least 100 variants in the 4.2.1 truth set of sample HG002.

Extended Data Table 1 | Single cell expression: CellRanger metric values for 10K cell and 1K cell libraries from the PBMC reference

CellRanger v7.0 Metric	Performance expectation	AVITI 10K cells	AVITI 1K cells
Valid barcodes	>90%	97.5%	97.5%
Reads mapped confidently to exonic regions	>50%	53.0%	53.8%
Read mapped confidently to transcriptome	>40%	74.7%	77.8%
Fraction reads in cells	>80%	95.5%	92.6%
Q30 bases in barcode	>85%	99.5%	99.5%
Q30 bases in RNA read	>75%	98.6%	98.8%
Mean reads per cell	>50,000	61,326	68,766
Median genes per cell	>1700	2,910	2,951
Total genes detected	N/A	23,863	29,679
Estimated number of cells	+/-20%	8,513	922

Extended Data Table 2 | Variant calling performance for HG002 on GIAB-HC regions

	Sensitivity	Precision	F1-Score
SNP	0.9939	0.9977	0.9958
Small indel	0.9928	0.9980	0.9954

Corresponding author(s): Michael Previte

Last updated by author(s): 3/7/2023

Reporting Summary

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- ☐ ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- ☒ ☐ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
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- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☒ ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

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Software and code

Policy information about [availability of computer code](#)

Data collection

Kinetic data for Figure 2A was collected using RQF3 Rapid Quench flow (Kintek corporation). Real-time measurements for Figure 2B-E were collected on an Olympus IX83 microscope equipped with 545 and 637 lines (Lumencor), Semrock brightline multiband laser filter set (LF405/488/532/635) containing matching quad band exciter, emitter and dichroic. Flow was induced by a syringe pump pulling reagents across an AVITI flow cell at a rate of 60 μ l/s. Prior to injection of reagents, real-time data was collected on an Andor sCMOS camera at 4 frames/s. All sequencing data was collected on the AVITI commercial instrument.

Data analysis

Kinetic data was analyzed and fit using conventional non-linear regression. All error bounds were propagated in the analysis and are reflected in figure 2 panel A. Reported k_{cat} and K_d were obtained by fitting to a hyperbolic equation using no constraints other than the error reported for each point. Primary analysis of the collected data was performed on the AVITI instrument according to similar steps described on Whiteford et al. (25) FASTQ were generated using the bases2fastq software toolkit (version 1.1.1). Tools and scripts supporting bioinformatic analysis of this manuscript can be found at the following repo located on github - <https://github.com/ElemBio/AvidityManuscript2023>. Single cell RNA was performed using Cell Ranger (version 7.0.1). Whole genome sequencing analysis was performed by first down-sampling the input FASTQ to 35X raw coverage (360,320,126, 2x150 input reads), and then aligning, de-duplicating and sorting using sentieon bwa (version 202112.02). The BAM was then used as input to Sentieon DNAscope (version 202112.02) in addition to a element specific ML model (SentieonDNAscopeModelElementBio0.3.model) to produce a VCF. Following alignment and variant calling, the variant calls were benchmarked using hap.py (version hap.py-0.3.14) to the NIST genome in a bottle truth set v4.2.1 across all regions to derive total error counts and F1 scores. To assess the accuracy of quality scores shown in Fig. 3, the aligned BAMs were processed using GATK BaseRecalibrator (version gatk4.4.2.0-0), and specifying publicly available known sites files to exclude human variant positions (HG002 NIST v4.2.1 bed/vcf,

1250C chase, sup2sig, reference hg38, chr1, chr18, the resulting bed files and generated figures were posted. To compute the mismatch percentage of AVITI, NovaSeq 6000, and NextSeq 2000 reads before and after homopolymers of length 12 or greater, a BED file provided by NIST genome-stratifications v3.0, containing 673,650 homopolymers of length greater than 11 was used to define the regions of interest for the homopolymer analysis (GRCh38_SimpleRepeat_homopolymer_gt11_slop5). Reads that overlapped these BED intervals (using samtools version 1.1.1) were selected for accuracy analysis. Reads with any of the following flags set were discarded (secondary, supplementary, unmapped or reads with mapping quality of 0). Reads were oriented in the 5' -> 3' direction, and split into 3 segments, preceding the homopolymer, overlapping the homopolymer, and following the homopolymer. The mismatch rate for each read-segment was computed, excluding N-calls, softclipped bases and indels. For example, if a 150 bp read (aligned on the forward strand) contains a homopolymer in positions 100-120, then the first 99 cycles were used to compute the error rate prior to the homopolymer, and the last 30 cycles were used to compute the error rate following the homopolymer. Reads were discarded if either the sequence preceding or following the homopolymer was less than 5bp in length (accounting for the GIAB slop used). All reads were then stacked into a matrix, according to their positional offset relative to the homopolymer, and error rate per pos-offset was computed. The average error rate was computed for avidity sequencing runs and for publicly available data from multiple SBS instruments, for comparison. The differences of mismatch percentages, across all BED intervals, between AVITI™ and NovaSeq were plotted in a histogram and examples showing various percentiles within the distribution were chosen for display via IGV.

The interval-error.tsv and offset-error.tsv files can be found in the following directory: https://github.com/Elombio/AvidityManuscript2023/tree/main/data/homopolymer-error/GRCh38_SimpleRepeat_homopolymer_gt11_slop5

To compute the mismatch percent difference between avidity sequencing and SBS across homopolymer lengths, the four GIAB supplied homopolymer bed files were combined, and duplicates were removed (4to6, 7to11, gt11, gt20), producing a new bed file representing all homopolymer of size 4 to inf. The box plot shows median, quartiles, and the whiskers are 1.5*IQR.

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The avidity sequencing data sets described in the manuscript are available for download via the AWS CLI using the following command:

```
aws s3 ls --no-sign-request s3://avidity-manuscript-data/
```

Samples and FASTQ have been accessioned in SRA under BioProject PRJNA869673.

Bioinformatic tools and scripts can be found on the following github repo: <https://github.com/Elombio/AvidityManuscript2023>

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Reporting on sex and gender

Population characteristics

Recruitment

Ethics oversight

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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Life sciences study design

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Sample size	Sequencing calibration studies were performed on 20 samples. Single cell studies were performed on multiple samples that generated consistent results, but a single example was used for this particular study. To determine k-mer errors, a million k-mers of each length were used to determine percent mismatch. For the homopolymer analysis, ~700,000 loci were used. For GiaB stratifications, we selected context classes with at least 100 variants.
Data exclusions	There was no data excluded (Filtered data is excluded from the sequencing runs).
Replication	We checked that all presented runs are representative by looking at no fewer than 20 sequencing runs. For analyses such as homopolymer and k-mer accuracy, sample size calculations are based on the number of relevant loci within a run. There were no failures to replicate.
Randomization	The study performed was validating first principles studies such as enzyme kinetics to validate the hypotheses of avidity chemistry, thus sample randomization would not be necessary. Sequencing data was performed on known samples and comparative metrics to known reference samples also obviates the need for randomization of the studies as the known reference samples are a widely known control.
Blinding	The study performed was validating first principles studies such as enzyme kinetics to validate the hypotheses of avidity chemistry, thus blind would not be necessary. Sequencing data was performed on known samples and comparative metrics to known reference samples also obviates the need for blind studies as the known reference samples are a widely known control.

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Materials & experimental systems

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
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<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern

Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

EXHIBIT 24



Element AVITITM System

FOR USE WITH

Element AVITI System, catalog # 880-00001
AVITI 2x75 Sequencing Kit CloudbreakTM, catalog # 860-00004
AVITI 2x150 Sequencing Kit Cloudbreak, catalog # 860-00003
AVITI 2x75 Sequencing Kit, catalog # 860-00002
AVITI 2x150 Sequencing Kit, catalog # 860-00001
AVITI Operating Software v2.0.0 or later

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CHAPTER 1

System Overview

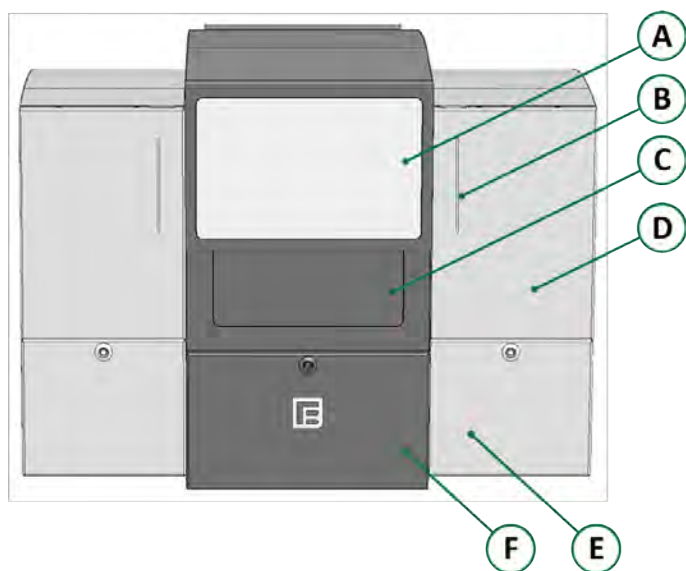
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Introduction

The Element AVITI System is a mid-throughput next-generation sequencing (NGS) system that amplifies and sequences DNA libraries. The system is divided into side A on the left and side B on the right. Each side operates independently so you can engage one side while the other is in use. A touchscreen monitor displays the AVITI Operating Software (AVITI OS).

This workflow guide provides a system overview and comprehensive instructions for safely operating and maintaining the instrument.

Figure 1: AVITI System overview



- A Touchscreen monitor
- B Lightbars for each side show system status
- C Nest bay contains the flow cells
- D Pump bays hold the pumps
- E Reagent bays hold reagents and wash solution
- F Waste bay holds waste bottles

Site Prep and Safety

The instrument does not contain any user-servicable parts. Software and interlocks prevent exposure to hazards, but using the AVITI System in an unspecified manner can compromise these protections. If performance is not to published specifications, contact Element Technical Support.

Before operating or maintaining the instrument, meet the site requirements detailed in the *Element AVITI System Site Prep Guide (MA-00007)*. Review the safety and regulatory information detailed in [Safety and Compliance on page 90](#).

System Compatibility

The AVITI System is compatible with single-strand DNA (ssDNA) libraries prepared with the Element Adept™ Library Compatibility Workflow, Element Elevate™ Library Prep Workflow, 16S LoopSeq™ for AVITI, or Amplicon LoopSeq for AVITI. LoopSeq for AVITI libraries include Elevate indexes and adapters and are therefore considered Elevate libraries.

The AVITI OS supports the chemistry combinations presented in the following table. The AVITI 2x75 Sequencing Kit and AVITI 2x150 Sequencing Kit use version 1 chemistry. Cloudbreak kits use Cloudbreak chemistry. To avoid mixing and matching components from different kit configurations and versions, AVITI OS validates the compatibility of the cartridge and flow cell provided in each sequencing kit.

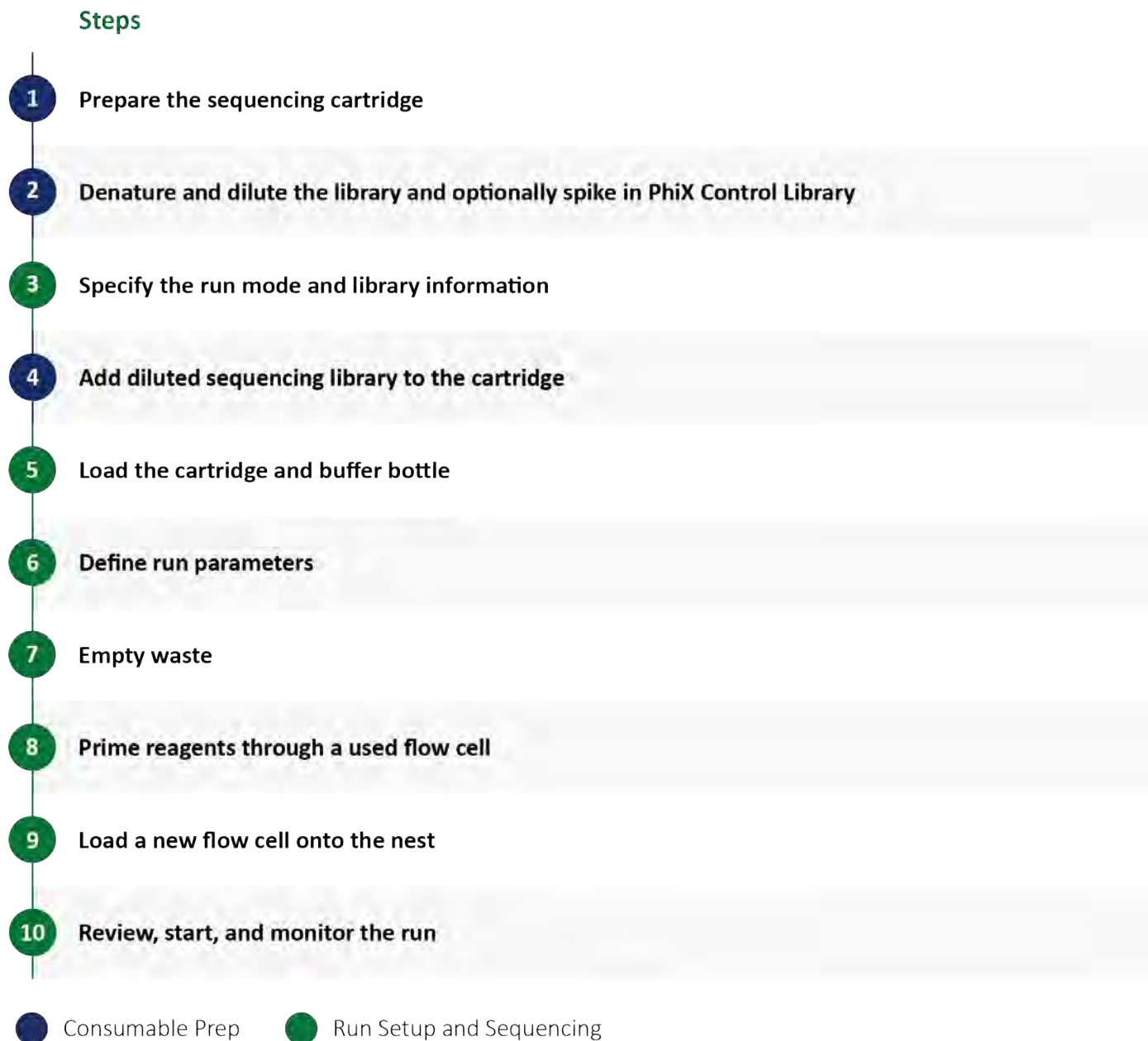
Table 1: Supported chemistry versions

Library Prep	Sequencing Kits*	Control Library	Custom Primers
Adept Workflow (v1.1 kits)	<ul style="list-style-type: none">• AVITI 2x75 Sequencing Kit• AVITI 2x150 Sequencing Kit	PhiX Control Library, Adept	Adept Custom Oligonucleotide Buffer Set
	<ul style="list-style-type: none">• AVITI 2x75 Sequencing Kit Cloudbreak• AVITI 2x150 Sequencing Kit Cloudbreak	PhiX Control Library, Adept	Adept Custom Primer Set Cloudbreak
Elevate Workflow	<ul style="list-style-type: none">• AVITI 2x75 Sequencing Kit• AVITI 2x150 Sequencing Kit	PhiX Control Library, Elevate	Not applicable
	<ul style="list-style-type: none">• AVITI 2x75 Sequencing Kit Cloudbreak• AVITI 2x150 Sequencing Kit Cloudbreak	Cloudbreak PhiX Control Library, Elevate	Not applicable

Workflow Summary

The following figure summarizes the steps to sequence on the AVITI System, including preparing diluted sequencing library and other consumables and setting up the run. Diluted sequencing library is a library or library pool at the appropriate volume and concentration for sequencing.

Figure 2: Overview of the sequencing workflow



Run Stages

AVITI OS generates a recipe based on run parameters entered during run setup. The recipe governs each stage of the run. A run is complete when the recipe is executed and primary analysis is done.

The following stages comprise a run:

- **Amplification**—Hybridizes the library to the flow cell and forms colonies, each containing multiple copies of the same sequence from the library.
- **Sequencing**—Performs each read in the run, including imaging and primary analysis.
- **Post-run wash**—Automatically flushes buffer from the sequencing cartridge through the fluidic system to remove salts and residual library.

Figure 3: Cloudbreak recipe for a paired-end run with indexing



Reads in a Run

Up to four reads comprise a run:

- **Index 1** sequences the Index 1 sequence and **Index 2** sequences the Index 2 sequence. A dual-index run performs both index reads, a single-index run performs Index 1 only, and a nonindexed run skips both index reads.
- **Read 1** sequences the forward strand of the DNA insert and is always required. Starting from the opposite end of the insert, **Read 2** sequences the reverse strand. A paired-end run performs both reads, including a paired-end turn before Read 2 to generate the complementary strand. A single-read run performs Read 1 only.

Number of Cycles

Read length is the total number of cycles a run includes. A 2 x 75 or 2 x 150 kit configuration indicates the number of cycles that sequence the DNA insert. You can distribute the total cycles depending on experimental design.

The optimal number of cycles to perform in a run depends on your experiment, but the software and chemistry prescribe a minimum and maximum. Read 1 requires at least five cycles to perform a run and at least 25 cycles to generate all run metrics. Elevate Cloudbreak libraries require at least four Index 1 cycles. The maximum number of cycles depends on configuration:

- A 2 x 75 kit sequences up to 184 cycles, supporting one 2 x 76 run with indexing and unique molecular identifiers (UMIs).
- A 2 x 150 kit sequences up to 334 cycles, supporting one 2 x 151 run with indexing and UMIs.

CHAPTER 2

Instrument Hardware

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AVITI System Hardware

The instrument is divided into side A on the left and side B on the right. Each side operates independently so you can engage one side while the other is in use. The middle of the instrument includes a glove-compatible touchscreen that displays the AVITI OS interface. Internally, a camera and four tube lenses image the flow cell in four channels.

Status Lights

The AVITI System includes two types of status lights: an exterior lightbar on each side of the instrument and an interior nest light in front of each nest. The lightbar colors indicate the current process and overall system status and the nest light colors indicate flow cell status.

Unless the system is initializing, each lightbar is side-specific. The color immediately changes when an error occurs. For a warning, the color changes after the run finishes.

Lightbar Colors

Color	Status
White fade	The system is initializing.
Solid white	The system is initialized and idle.
Solid blue	Run or wash setup is in progress.
Blue fade	The system is priming, sequencing, or washing.
Solid orange	The system experienced a warning.
Solid red	The system experienced an error or run failure.

Nest Light Colors

Color	Status
Blue	The flow cell is present and ready to be unloaded.
Green	The flow cell is properly loaded and ready for priming, sequencing, or washing.
Red	The flow cell is improperly loaded: the lid is open or the nest is empty.
None	The flow cell is present but is not ready to be unloaded.

Exterior Shells

Exterior shells enclose the instrument to maintain internal temperatures, exclude dust and other external elements, and protect operators from exposure to lasers, mechanical moving parts, and other internal hazards. For more information, see [Safety and Compliance on page 90](#).

Instrument Bays

Instrument bays hold consumables and accessories for runs and washes. Each side includes a dedicated reagent bay and a dedicated pump bay. Bays in the middle of the instrument hold flow cells and waste for both sides.

Lighting illuminates the interior of each bay. During a run, AVITI OS locks all doors except the pump bay doors to protect against lasers, mechanical moving parts, and other hazards.

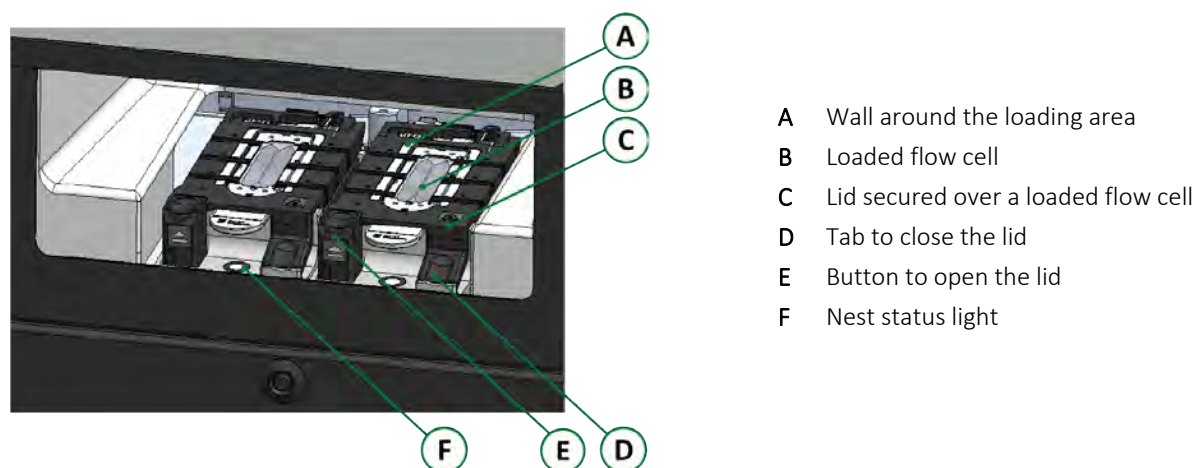
CAUTION
Do not place items on top of the instrument or open doors. The doors can support the weight of run and wash components but leaning on or bumping into a door can damage the instrument.

Nest Bay

The nest bay includes two nests, one for each side. Each nest holds one flow cell secured with a lid. A button unlatches and opens the lid to a 40° angle. A tab closes the lid and secures the flow cell.

To guide placement of the flow cell onto the nest, a wall encircles the loading area. Three silver pins fit into three corresponding holes on the flow cell cartridge, ensuring proper alignment and seating. An automated door encloses the bay.

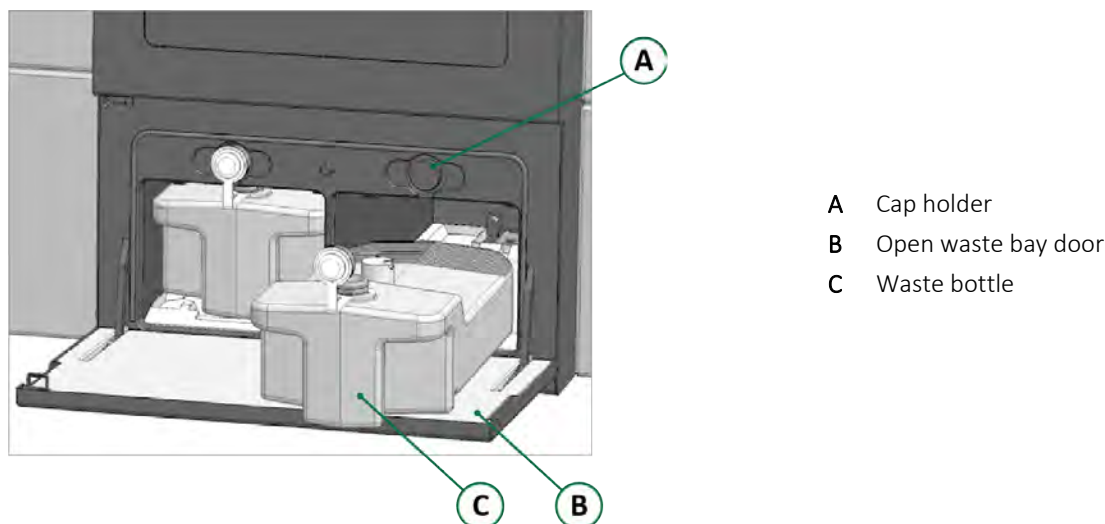
Figure 4: Nest bay with loaded flow cells



Waste Bay

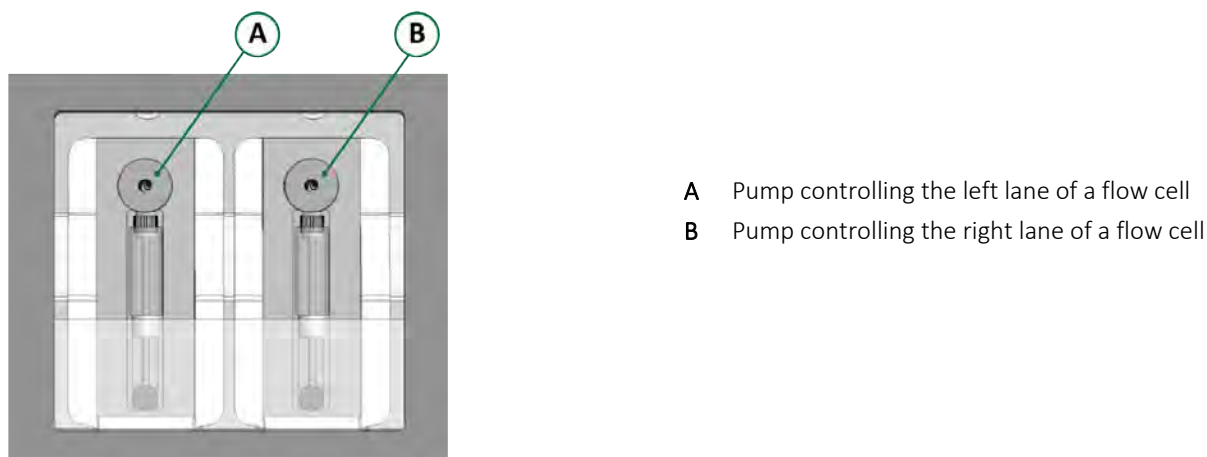
The waste bay holds two waste bottles, one for each side. Two threaded cap holders above the waste bay secure the transport cap tethered to each waste bottle to keep the caps clear of the door.

A sealed tray built into the bottom of the waste bay collects spills and leaks for cleaning and directs liquid to the front of the instrument. Welding prevents liquid from entering the area behind the waste bay. During run or wash setup, a sensor confirms the waste bottle is present and empty and allows the run or wash to proceed. Another sensor detects any spills.

Figure 5: Open waste bay with waste bottles

Pump Bays

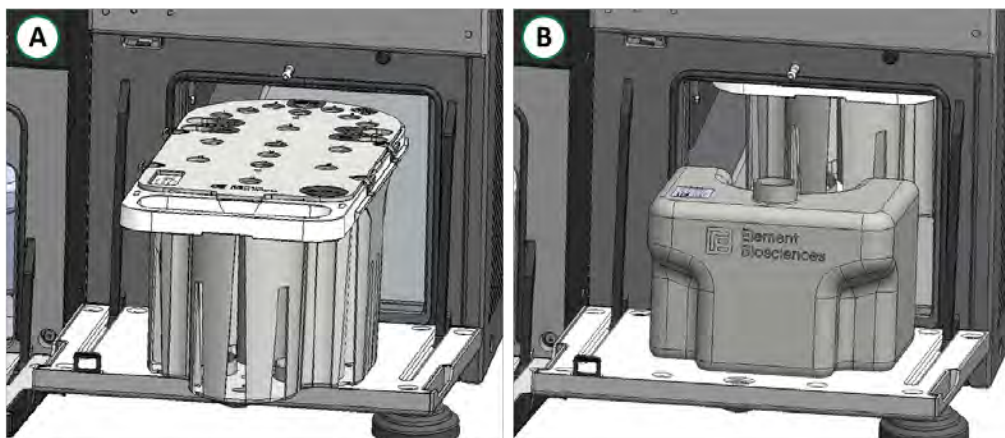
Each pump bay contains two pumps that control the flow of liquid. The left pump pulls fluid through the left lane of the flow cell and the right pump pulls fluid through the right lane. Keep the pump bay doors, which allow service access, closed during normal operation and maintenance.

Figure 6: Pumps in a pump bay

Reagent Bays

Each reagent bay holds a buffer bottle and sequencing basket that contains a cartridge or a wash tray, depending on whether the system is sequencing or washing. Keep the reagent bay doors closed to maintain the refrigeration, which chills reagents.

When priming starts, sippers descend into the bay, pierce the foil seals covering the cartridge wells, and aspirate reagents from the bottom of each well. The sippers continue to aspirate reagents throughout the run. Functioning similarly for a wash, the sippers aspirate wash solution instead of reagents.

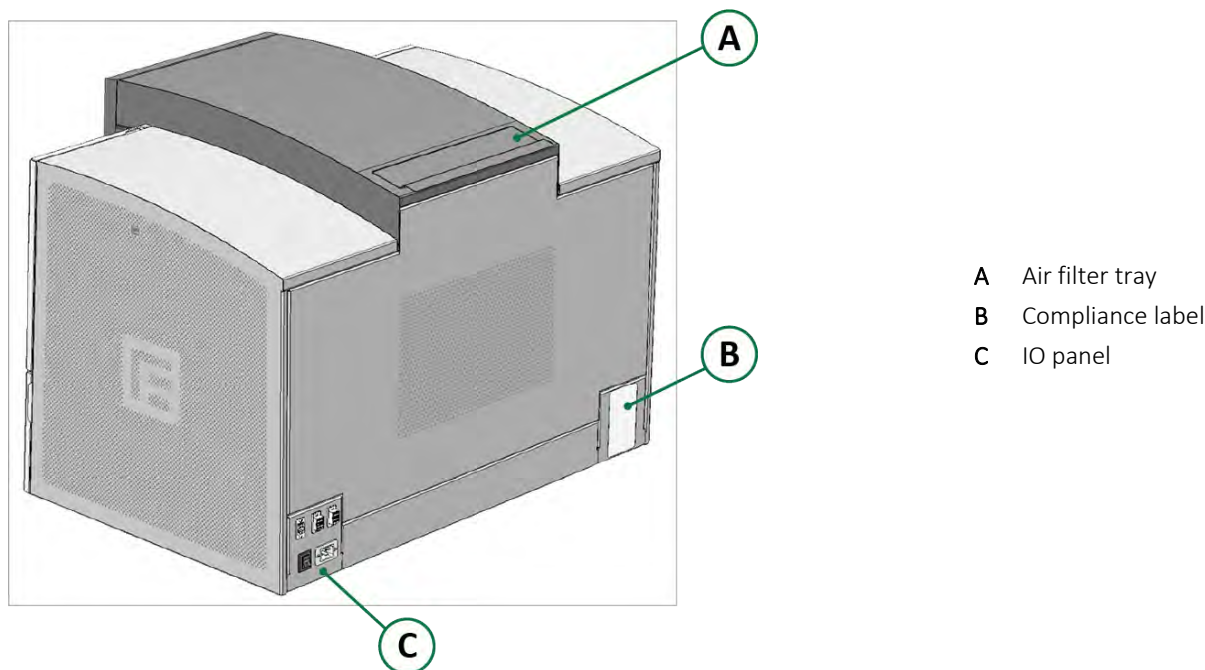
Figure 7: Open reagent bay with reagents

- A Loading a basket and cartridge into the reagent bay
- B Loading a buffer bottle behind the basket

Back Panel

The back panel includes the air filter tray and input and output (IO) panel. A compliance label displays regulatory symbols for regulatory compliance, the instrument serial number, and electrical specifications. For more information on labeling, compliance, declarations, and certifications, see [Safety and Compliance on page 90](#).

Figure 8: Back panel of the instrument



Air Filter Tray

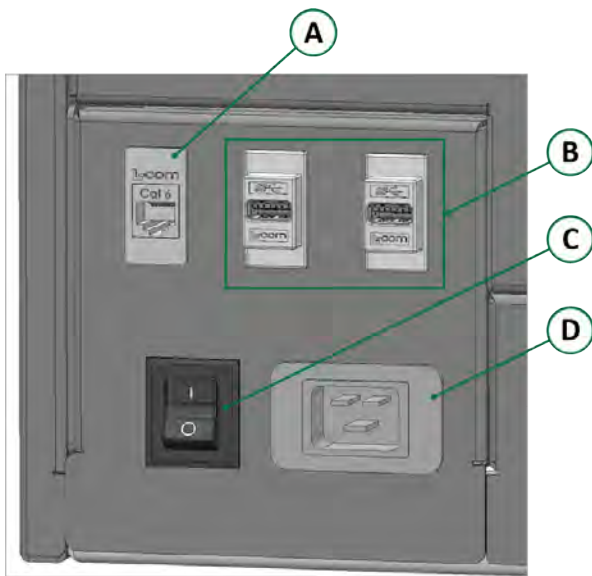
Air enters the instrument through a disposable air filter constructed of pleated paper. The air filter is rated MERV 9, which keeps dust out of the instrument but does not filter smoke or particles < 3 microns. Keeping aerosol and particulate sources away from the instrument extends filter life.

A tray that lifts out of the top of the instrument contains the air filter and facilitates easy replacement. For instructions, see [Replace the Air Filter on page 67](#).

Input and Output Panel

An IO panel on the back of the instrument groups connections and the power switch. A Category 6 (Cat6) Ethernet port connects an Ethernet cable and a power entry module connects the power cord. When connecting the instrument to power, use only the power cord that shipped with the instrument.

The IO panel also includes two USB 3.0 ports to connect a mouse, keyboard, or drive for transferring files. Side B includes a third USB 3.0 port. A USB drive that transfer files to or from the instrument must be in **FAT32 format**.

Figure 9: Power and Ethernet on the IO panel

- A Cat6 Ethernet port
- B USB 3.0 ports
- C Power switch in the on position
- D Power entry module

CHAPTER 3

Software and Analysis

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AVITI Operating Software

AVITI OS is the main software installed on the AVITI System. The Home screen functions as a system dashboard, displaying the status of each side with features to start runs and washes and monitor sequencing runs.

Figure 10: Home screen components



Home Screen Views






The Home screen includes the following views:

- **Overview**—Displays a general status and past sequencing run time for the side or summarizes an active run or wash.
- **Details**—Displays metrics for an active run. When a run starts, AVITI OS automatically switches to this view.
- **History**—Preserves metrics from the last run. When no run or wash is active, this view is available.

The Overview, Details, and History buttons update the Home screen view. You can switch between details and overview or history and overview.

Taskbar Icons

A taskbar at the top of the Home screen provides the following icons. The Settings and Notifications icons each open a unique workspace. Keyboard, USB Drive, and User access additional features and functions.

Icon	Name	Function
	USB Drive	View a list of USB drives connected to the instrument and safely disconnect a USB drive from the instrument.
	Settings	View system information and configure settings. See Settings on page 21 .
	Notifications	Review run, wash, instrument, or software notifications and take the indicated action. See Notifications on page 22 .
	Keyboard	Open a keyboard that accepts touchscreen input.
	User	Open the User menu. Alternatively, this icon displays initials.

Run Start Options

AVITI OS includes the following options for starting a run:

- **Single start**—Set up and start a run on one side of the instrument.
- **Dual start**—Concurrently set up and start runs on both sides of the instrument.
- **Flexible start**—Set up and start a run on one side of the instrument. While the run is in progress, set up and start a run on the other side so sequencing proceeds asynchronously.

AVITI OS allows sequencing with a version 1 kit on one side and a Cloudbreak kit on the other. Because the sides share a camera, this setup increases the duration of the Cloudbreak run.

Run Setup Screens

When you initiate a run, AVITI OS guides you through a series of run setup screens. Each screen indicates how far along setup is and displays callouts to guide consumable loading. Workflow-specific checkboxes ensure the readiness of each consumable.

AVITI OS unlocks the reagent and waste bay doors at the appropriate steps. Closing a door validates consumable or accessory presence and scans consumable barcodes. After the waste step, priming starts automatically. Priming prepares reagents for delivery and pumps air and reagents through a used flow cell and the fluidic tubes, preventing contamination between runs.

Wash Setup Screens

Initiating a wash opens a series of wash setup screens that guide you through setting up a maintenance, standby, or recovery wash. Wash setup functions similar to run setup, but closing the door validates the wash tray presence.

Flexible Start

Flexible start safely pauses the active run and initiates a sequencing run on the other side of the instrument. When setting up the second run, AVITI OS finds a safe pause point before proceeding. Pausing the first run typically takes several minutes but can take as long as ~2 hours, depending on the run stage.

When you initiate flexible start, AVITI OS displays the expected wait time. AVITI OS also includes options to cancel flexible start and resume the active run.

Settings

Settings includes configurable and read-only settings that control the instrument profile and system connections. AVITI OS divides the settings among the following tabs. The Network and Storage tabs include connectivity indicators. The User tab is unique to offline systems and online systems with local authentication.

- **About**—Displays software and instrument information:
 - » AVITI OS version and the last license acceptance date
 - » AVITI System name, serial number, available local storage, and compute ID
 - » Updates available for system firmware and software
- **General**—Controls the system name, displays telemetry and elevation settings, and exports log files from offline systems.
- **Network**—Controls network and internet connections for the system.
- **Storage**—Lists storage connections with settings for adding and managing storage connections.
- **User**—Provides password management for offline systems and online systems with local authentication.






NOTE

Compute ID is a unique code for the integrated circuit that identifies the instrument computer.



Network Status

The Network tab displays the following icons, which indicate the status of the network connection. An additional Indicator appears on the tab to show internet connectivity.

Icon	Network Status
	Connected
	Local internet only
	Disconnected

Storage Status





The Storage tab displays the following icons, which indicate the status of the storage connections. An additional Indicator appears on the tab to show storage connectivity.

Icon	Storage Status
	At least one storage connection
	No storage connection

Notifications



Notifications display system messages across three tabs: General, Side A, and Side B. Expand a notification to see the message, date, and time.

Table 2: Types of notifications

Notification	Icon	Description	Action
Success		A run or wash completed successfully.	Acknowledge successful completion.
Information		The software is ready to be updated to a new version.	Acknowledge the update.
Warning		The system requires your attention, but you can continue operation.	Acknowledge the warning and resolve it by the indicated date.
Error		The system has malfunctioned and requires action to proceed.	Follow the onscreen prompt.

Unread Notifications

Notifications include badges that indicate the number of unread messages. Checkboxes mark notifications as read or unread. Marking a notification as read can reset the status lights on that side of the instrument.

Icon	Name	Action
	Mark as read	Mark the selected notifications as read.
	Mark as unread	Mark the selected notifications as unread.

Filtering and Sorting

Notifications include filters with sorting from newest to oldest or oldest to newest.

Filter	Description
All	View all messages on the selected tab.
Read	View only read messages on the selected tab.
Unread	View only unread messages on the selected tab.

Signing In and Out

Signing in to AVITI OS requires the email address and password for your organization. The first time you sign in to AVITI OS after instrument installation or an update, you must accept the license agreement. A Logout option on the User menu signs you out.

If requested, Element can enable local authentication mode for an online system. This feature assigns a fixed user name and user-defined password to sign in.

Primary Analysis

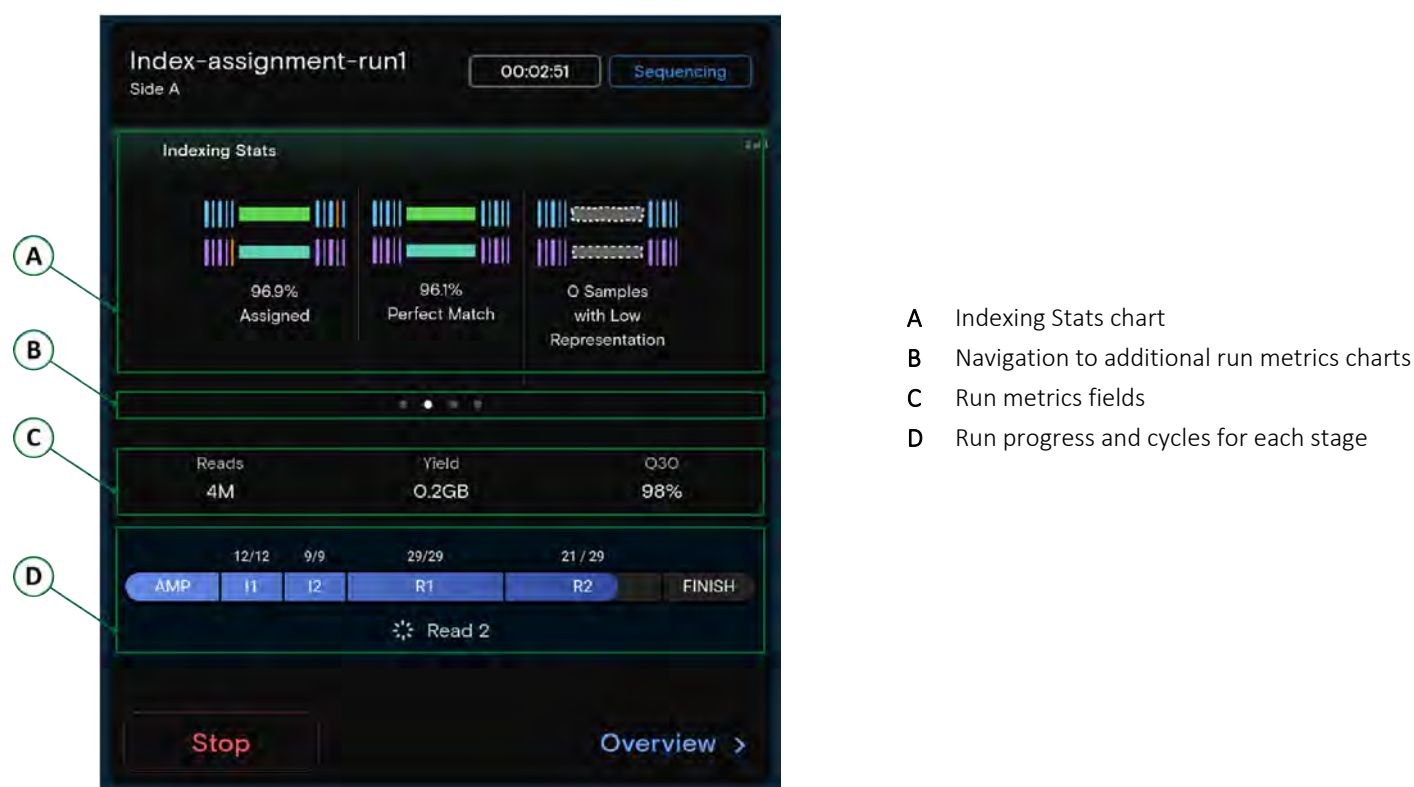
Onboard primary analysis software calls bases, assigns quality scores (Q-scores), and generates run metrics. The software extracts and corrects intensities from images to call a base, then assigns a Q-score to the base to indicate confidence in the call.

Run Monitoring

During a run, the Details view displays primary analysis-generated run metrics that monitor overall run health and progress. As the run progresses, metrics appear followed by regular updates. The metrics are included in the run output and remain onscreen until you set up a new sequencing run.

Run metrics are divided into Reads, Yield, and Q30 fields and a series of charts that you can cycle through: % Q30, Indexing Stats, PhiX Error, and Run Configuration. PhiX Error applies only to runs that include a spike in of PhiX Control Library. Indexing Stats apply only to runs that include a run manifest with 1–364 index pairs.

Figure 11: Details view for a run on side A



Run Metrics

The following table lists the run metrics displayed on the Home screen. Taken together, metrics on the Indexing Assignment tab estimate index quality and provide an early alert to potential problems with the run manifest or library.

Metric	Tab	Description
Reads	Not applicable	The number of reads the run is generating.
Yield	Not applicable	The amount of sequencing data in gigabases the run is expected to produce.
Q30	Not applicable	The percentage of base calls \geq Q30. Q30 indicates a 1 in 1000 possibility of an incorrect base.
% Q30	Q30	A chart indicating the percentage of base calls in each Read 1 and Read 2 cycle that have a Q-score \geq Q30.
Assigned	Indexing Assignment	The percentage of reads with indexes assigned.
Perfect Match	Indexing Assignment	The percentage of reads with indexes assigned that have a perfect index match.
Samples with Low Representation	Indexing Assignment	The number of samples with $< 10,000$ colonies assigned.
PhiX error rate	PhiX Error Rate	The percentage of control reads in each Read 1 and Read 2 cycle that do not align to PhiX Control Library.

Run Output and Storage

The output of a run is the run folder, which contains bases files and other run data. A storage connection transfers the run folder from the instrument to your storage location, which can be local or in the cloud. For more information, see [Storage Connections on page 69](#).

After a run, use Bases2Fastq Software to perform demultiplexing and convert the bases files into FASTQ files for secondary analysis with third-party software of your choice. Bases files contain genomic data and are the primary output of a run.

Figure 12: Data processing components



Run Folder

A run folder is named for the run name and contains the following files, which are organized at the root directory or in a subfolder. Braces around a file name component indicate variability. For example, the value for {tile} depends on the applicable tile number.

File	Directory and File Name	Description	Quantity
Alignment	Root/Alignment/{read}_{tile}.aln	Binary files that indicate which colonies align to PhiX Control Library	One per tile per read
Bases	Root/BaseCalls/{tile}/{read}_{tileName}_C{cycle:000}.bases.gz	Binary files that contain base calls and associated Q-scores	One per read, tile, and cycle
Filter	Root/Filter/{tile}.filter	Binary files that contain the filter status for each colony	One per tile
Index assignment	Root/IndexAssignment.csv	Comma-separated values (CSV) file that records the results of onboard demultiplexing	One per run
Location	Root/Location/{tile}.loc	Binary files that identify colony locations on the flow cell	One per tile
Run manifest	Root/RunManifest.csv	CSV file that records biological sample information and analysis settings	One per run
	Root/RunManifest.json	JavaScript Object Notation (JSON) file reserved for Element processes	One per run
Run parameters	Root/RunParameters.json	JSON file that records information about the run configuration	One per run
Run statistics	Stats/AvitiRunStats.json	JSON file that records run metrics	One per run

File	Directory and File Name	Description	Quantity
Run uploaded	Root/RunUploaded.json	Empty JSON file that is the last file transferred and marks run completion	One per run

Run Manifest

The run manifest stores the information for the contents of a sequencing run and analyzing the results. The run manifest includes demultiplexing settings, FASTQ file settings, and a list of samples with any corresponding index sequences.

Sequencing indexed libraries requires preparing a run manifest for the run. When a run does not include a run manifest, AVITI OS generates a default run manifest that assigns all reads to one sample during FASTQ generation. Thus, demultiplexing indexed libraries with a default run manifest is ***not possible***. For help preparing a run manifest, see the *Run Manifest Workflow Guide (MA-00011)*.

Local Disk Storage

Because the system software transfers runs to off-instrument storage locations, local disk storage is intended only for temporary storage. Accordingly, the instrument hard drive has sufficient space to store two runs and start an additional two runs.

When you initiate run setup, AVITI OS checks whether the system has sufficient space to support the run. If AVITI OS indicates that the system does not have sufficient space, contact Element Technical Support.

Telemetry

Separate from the transfer of genomic data to your storage location, **which Element cannot access**, telemetry sends instrument health data to Element. These data help support maintenance and troubleshooting and do not include any confidential information.

Telemetry is limited to the following data:

- **Software metrics**—Software and firmware versions, CPU and memory metrics, and the instrument serial number, ID, and name. These data are communicated as part of regular telemetry events.
- **Hardware metrics**—Data on motors, fans, lasers, and other instrument hardware, which helps Element understand the probable condition of select hardware components.
- **System logs**—Routine logs the system generates when idle or running. The logs include power cycle times, errors, internal communications, and the status of internal services.
- **Primary analysis metrics**—Sequencing metrics, including data for Q30 scores, error rates, and index assignment metrics. The index assignment data exclude sample names.
- **Run information**—Data communicated for a run, including run name and ID, run side, run start and end dates and times, run type (sequencing or washing), consumable information, and the number of cycles per read. The data exclude run descriptions.
- **Run logs**—Run-specific information from a subset of system logs. Data include recipe execution, the timing of run steps, and communications between software, firmware, and hardware.

CHAPTER 4

Sequencing Materials

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Sequencing Kits

A sequencing kit provides the flow cell and reagents for a run. Performing a run on one side of the instrument requires one sequencing kit in a 2 x 75 or 2 x 150 configuration. A dual or flexible start run requires two kits. To ensure the compatibility of run components, see [System Compatibility on page 6](#).

When using a sequencing kit and other reagents, always wear personal protective equipment (PPE): a lab coat, powder-free disposable gloves, and protective goggles. Review the safety data sheets (SDS) for chemical properties. The SDS inform safety, disposal, and hazards for your region and are available at go.elembio.link/sds.

Sequencing Kit Contents and Storage

Each sequencing kit is single-use and packaged in two boxes. The following tables list the kit contents and storage requirements. When you receive your kit, promptly store the components at the proper temperatures.

**CAUTION**

The cartridge contains light-sensitive reagents. Keep the cartridge packaged until use and **protect from light**.

AVITI 2x75 Sequencing Kit

Component	Quantity	Shipping Temperature	Storage Temperature
AVITI 2x75 Sequencing Cartridge	1	-25°C to -15°C	-25°C to -15°C
AVITI Flow Cell Cartridge	1	Room temperature	2°C to 8°C
AVITI Oligonucleotide Set	1	-25°C to -15°C	-25°C to -15°C
AVITI Universal Wash Buffer	1	Room temperature	Room temperature
Library Loading Buffer	2	-25°C to -15°C	-25°C to -15°C

AVITI 2x150 Sequencing Kit

Component	Quantity	Shipping Temperature	Storage Temperature
AVITI 2x150 Sequencing Cartridge	1	-25°C to -15°C	-25°C to -15°C
AVITI Flow Cell Cartridge	1	Room temperature	2°C to 8°C
AVITI Oligonucleotide Set	1	-25°C to -15°C	-25°C to -15°C
AVITI Universal Wash Buffer	1	Room temperature	Room temperature
Library Loading Buffer	2	-25°C to -15°C	-25°C to -15°C

AVITI 2x75 Sequencing Kit Cloudbreak

Component	Quantity	Shipping Temperature	Storage Temperature
AVITI 2x75 Cartridge Cloudbreak	1	-25°C to -15°C	-25°C to -15°C
AVITI Flow Cell Cloudbreak	1	Room temperature	2°C to 8°C
Adept Primer Set Cloudbreak	1	-25°C to -15°C	-25°C to -15°C
AVITI Universal Wash Buffer	1	Room temperature	Room temperature
Library Loading Buffer	2	-25°C to -15°C	-25°C to -15°C

AVITI 2x150 Sequencing Kit Cloudbreak

Component	Quantity	Shipping Temperature	Storage Temperature
AVITI 2x150 Cartridge Cloudbreak	1	-25°C to -15°C	-25°C to -15°C
AVITI Flow Cell Cloudbreak	1	Room temperature	2°C to 8°C
Adept Primer Set Cloudbreak	1	-25°C to -15°C	-25°C to -15°C
AVITI Universal Wash Buffer	1	Room temperature	Room temperature
Library Loading Buffer	2	-25°C to -15°C	-25°C to -15°C

Kit Components

The components in a 2 x 75 and 2 x 150 kit are the same except for the sequencing cartridge. A 2 x 150 cartridge includes a greater volume of reagents to support more cycles. For more information, see [Number of Cycles on page 9](#).

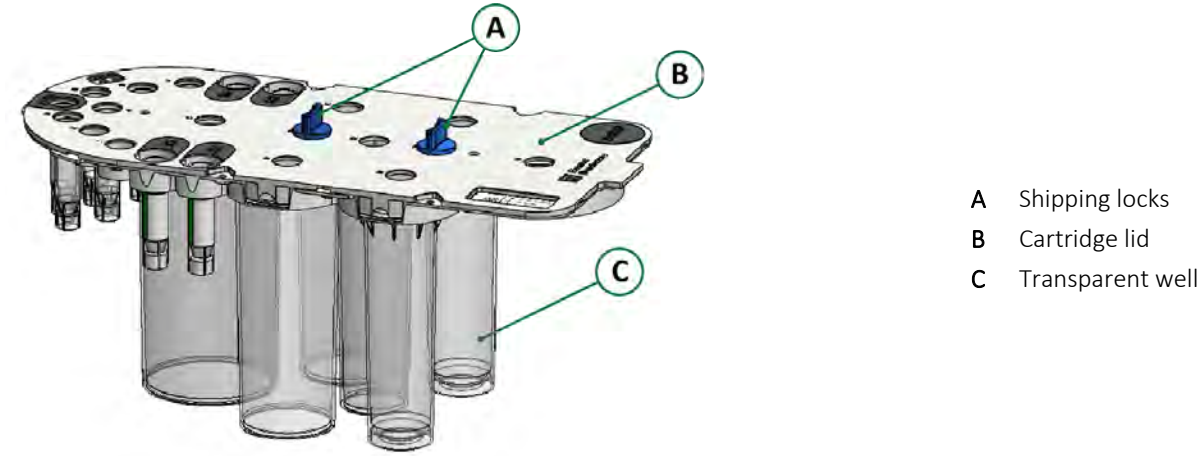
Each kit component includes a barcode label for tracking and validation purposes.

Sequencing Cartridge

A sequencing cartridge packages the amplification, paired-end, indexing, and cycling reagents and a post-run wash buffer into a convenient container that facilitates reagent preparation, loading, and disposal. A lid covers the cartridge to retain and label the reagents. Two shipping locks secure the lid and remain intact until run setup.

Each reagent occupies a foil-sealed well that is transparent to allow visual inspection. The Library well is reserved for diluted sequencing library. The I1, I2, R1, and R2 wells contain replaceable primers. Some wells are intentionally empty. For a cartridge map, see [Discard the Cartridge and Bottle on page 55](#).

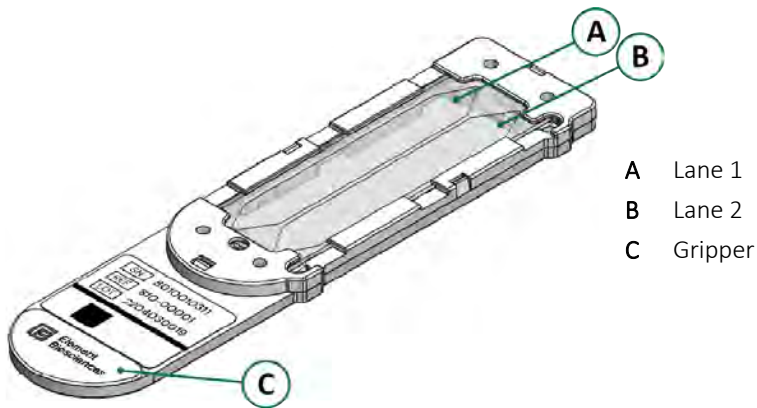
ELEMENT BIOSCIENCES CONFIDENTIAL, PRERELEASE MATERIAL



Flow Cell

The flow cell is a two-lane glass substrate encased in a plastic cartridge. The outlet end of the cartridge extends into a handle with a gripper for safe handling.

Proprietary surface chemistry coats the flow cell and enables polony generation and sequencing. Library and reagents enter the flow cell through inlet ports at the top of each lane, saturating the surface and exiting as waste through outlet ports at the bottom.



Adept Primer Set

The primers in the I1, I2, R1, and R2 wells of the cartridge support Elevate libraries. Sequencing Adept libraries requires replacing the prepackaged primers with the tubes from the AVITI Oligonucleotide Set or Adept Primer Set Cloudbreak.

Table 3: Primer set contents

Primer	AVITI Oligonucleotide Set	Adept Primer Set Cloudbreak
Index 1	Adept Index 1 (I1) Primer	Adept Index 1 (I1) Primer Cloudbreak

Primer	AVITI Oligonucleotide Set	Adept Primer Set Cloudbreak
Index 2	Adept Index 2 (I2) Primer	Adept Index 2 (I2) Primer Cloudbreak
Read 1	Adept Read 1 (R1) Primer	Adept Read 1 (R1) Primer Cloudbreak
Read 2	Adept Read 2 (R2) Primer	Adept Read 2 (R2) Primer Cloudbreak

Loading and Wash Buffers

A sequencing kit includes multiple loading and wash buffers. The loading and wash buffers are packaged separately. Instrument Wash is included in the cartridge.

Table 4: Buffer types

Buffer	Packaging	Description
Library Loading Buffer	Tube	Dilutes the experimental library and optional PhiX Control Library to the target loading concentration before a run
AVITI Universal Wash Buffer	Buffer bottle	Flushes excess reagents from the flow cell between certain chemistry steps
Instrument Wash	Cartridge	Serves as the wash solution for the automatic post-run wash

Controls and Custom Primers

In addition to the sequencing kits, Element offers PhiX Control Library and custom primer sets. These components supplement the sequencing kits, providing additional reagents for runs that include sequencing controls and custom primers.

PhiX Control Library

PhiX Control Library is a color-balanced, ready-to-use library that functions as a positive control and adds diversity to low-complexity libraries. Each type of PhiX Control Library includes unique index sequences, which the *Run Manifest Workflow Guide (MA-00011)* lists.

Table 5: Control library types

Type	Format	Stock Concentration	Shipping and Storage Temperature
PhiX Control Library, Adept	Circular	1 nM	-25°C to -15°C
PhiX Control Library, Elevate	Circular	1 nM	-25°C to -15°C
Cloudbreak PhiX Control Library, Elevate	Linear	1 nM	-25°C to -15°C

Custom Primer Sets

A custom primer set provides read-specific buffers for preparing custom primers to sequence Adept libraries. The buffers are packaged in tubes that fit the I1, I2, R1, and R2 wells of the sequencing cartridge.

Table 6: Custom primer set contents

Custom Primer Set	Buffers	Shipping and Storage Temperature
Adept Custom Oligonucleotide Buffer Set	Adept Custom Index 1 (I1) Buffer	-25°C to -15°C
	Adept Custom Index 2 (I2) Buffer	-25°C to -15°C
	Adept Custom Read 1 (R1) Buffer	-25°C to -15°C
	Adept Custom Read 2 (R2) Buffer	-25°C to -15°C
Adept Custom Primer Set Cloudbreak	Adept Custom Index 1 Buffer, Index First (I1)	-25°C to -15°C
	Adept Custom Index 2 Buffer, Index First (I2)	-25°C to -15°C
	Adept Custom Read 1 Buffer, Index First (R1)	-25°C to -15°C
	Adept Custom Read 2 Buffer, Index First (R2)	-25°C to -15°C

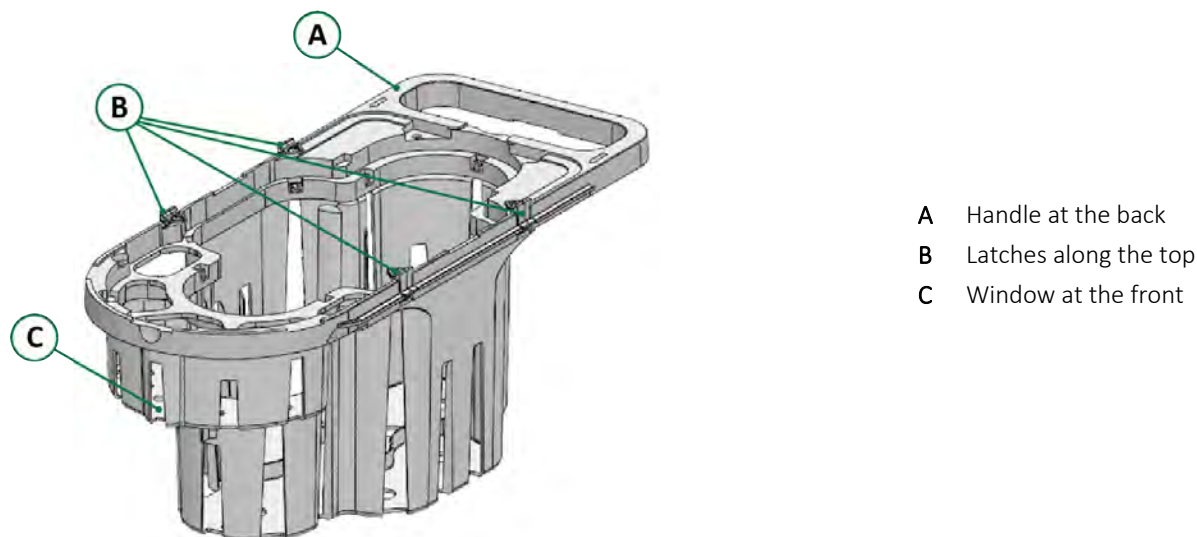
Reusable Accessories

Sequencing baskets, wash trays, and waste bottles support run setup and washes while minimizing waste. These accessories are reusable but require periodic replacement.

Sequencing Basket

A sequencing basket protects the cartridge during a run. The back of the basket extends into a handle with arrows that indicate the loading direction. Clips along the top of the basket secure the cartridge. The curved area under the handle accommodates the buffer bottle, which is loaded into the reagent bay behind the basket. A window at the front of the basket enables library inspection.

Figure 13: Sequencing basket features

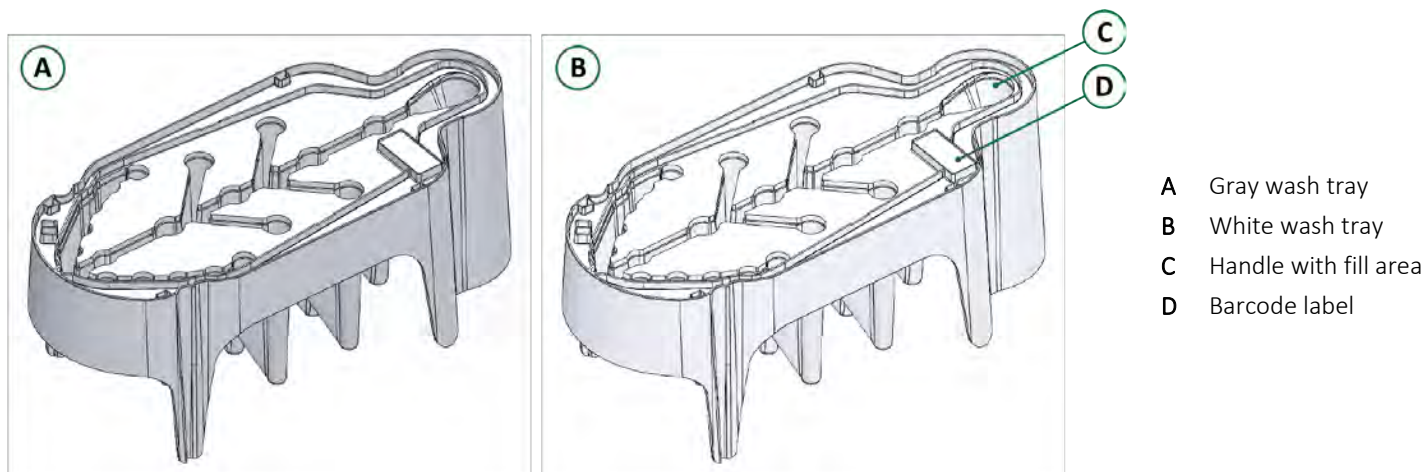


Wash Trays

The AVITI System includes two types of wash trays, each dedicated to different wash solutions:

- AVITI Wash Tray 1, Gray, for use with Wash 1 Solution.
- AVITI Wash Tray 2, White, for use with Wash 2 Solution and nuclease-free water.

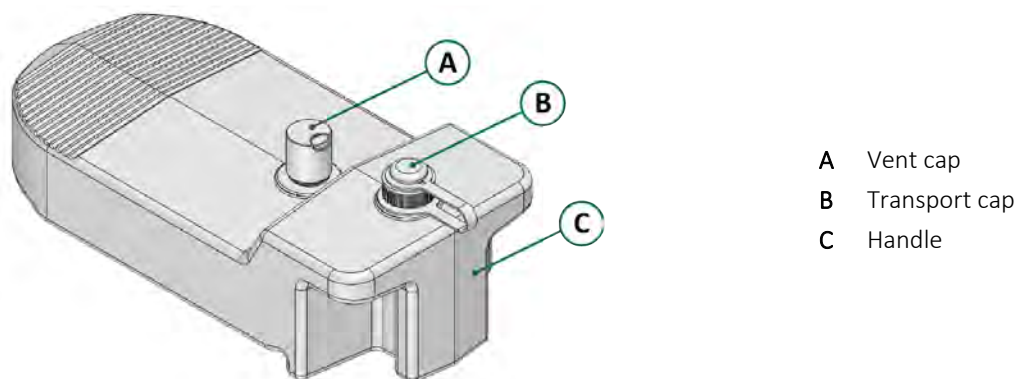
The back of a wash tray forms a handle with a fill area for adding wash solution. Interior fill lines indicate approximate volumes and an overflow wall contains any wash solution that exceeds the 800 ml maximum fill volume. Each tray includes a water-proof barcode label for validation purposes and a clear lid to prevent spills during transportation.



Waste Bottle

A waste bottle collects spent reagents and library. The maximum capacity of 3.2 L per bottle is sufficient to contain all waste from one run on one side of the instrument. Attaching a funnel to the waste receptacle prevents splashing and spills when emptying waste, particularly for smaller receptacles.

Two caps on top of the waste bottle contain waste: a transport cap and a vent cap. The transport cap is tethered and sits above the handle, sealing the bottle for transportation of waste. The vent cap sits lower on the bottle. Opening the vent cap when emptying waste improves the flow of liquid. Ridges on the back of the bottle and a handle at the front facilitate handling.



User-Supplied Materials

Instrument operation and maintenance use the materials listed in the following tables. To ensure compatibility of Element run components, see [System Compatibility on page 6](#).

Library prep requires a separate set of materials, which are listed in the Adept and Elevate workflow guides and materials lists.

User-Supplied Sequencing Consumables

Supplier	Consumable	Catalog #
General lab supplier	0.2 M Tris-HCl, pH 7.0	Not applicable
	1 N NaOH	Not applicable
	10 mM Tris-HCl, pH 8.0 with 0.1 mM EDTA	Not applicable
	Filtered pipette tips	Not applicable
	Low TE buffer	Not applicable
	Nuclease-free water	Not applicable
Element Biosciences	Any sequencing kit: <ul style="list-style-type: none">• AVITI 2x75 Sequencing Kit• AVITI 2x150 Sequencing Kit• AVITI 2x75 Sequencing Kit Cloudbreak• AVITI 2x150 Sequencing Kit Cloudbreak	The corresponding catalog #: <ul style="list-style-type: none">• 860-00002• 860-00001• 860-00004• 860-00003
	[Optional] Either custom primer set: <ul style="list-style-type: none">• Adept Custom Oligonucleotide Buffer Set• Adept Custom Primer Set Cloudbreak	The corresponding catalog #: <ul style="list-style-type: none">• Catalog # 820-00008• Catalog # 820-00009
	[Optional] Any control library: <ul style="list-style-type: none">• PhiX Control Library, Adept• PhiX Control Library, Elevate• Cloudbreak PhiX Control Library, Elevate	The corresponding catalog #: <ul style="list-style-type: none">• Catalog # 830-00004• Catalog # 830-00002• Catalog # 830-00017
Eppendorf	DNA LoBind Tubes, 2 ml	Catalog # 022431048

User-Supplied Maintenance Consumables

Supplier	Consumable	Catalog #
General lab supplier	Microfiber cloths	Not applicable
	Nuclease-free water	Not applicable
	Polyurethane foam-tip swabs with plastic handles	Not applicable
	Serological pipettes	Not applicable
	Simple Green All-Purpose Cleaner	Not applicable*
Element Biosciences	Air Filter, MERV 9	Catalog # 350-00065
MilliporeSigma	Sodium hypochlorite solution, reagent grade, 4.00–4.99%, 500 ml	Catalog # 239305*
Thermo Fisher Scientific	Either bottle: <ul style="list-style-type: none"> • Nalgene HDPE Heavy-Duty Bottles with Closure, 2 L • Nalgene Large Narrow-Mouth LDPE Bottles, 2 L 	The corresponding catalog #: <ul style="list-style-type: none"> • 2125-2000PK* • 2202-0005PK*
VWR	Alcohol Prep Pads, 70% isopropyl alcohol	Catalog # 95041-712*
	Tween 20, reagent grade, 1 L	Catalog # 97062-332*

* Or equivalent

User-Supplied Equipment and Accessories

Supplier	Consumable	Catalog #
General lab supplier	Freezer, -25°C to -15°C	Not applicable
	Ice bucket	Not applicable
	Mini centrifuge	Not applicable
	Pipette controller	Not applicable
	Pipettes, single-channel	Not applicable
	Refrigerator, 2°C to 8°C	Not applicable
	Tub for water baths	Not applicable
	Vortex mixer	Not applicable
	[Optional] FAT32 USB drive	Not applicable
Element Biosciences	AVITI Sequencing Basket	Catalog # 890-00025
	AVITI Wash Tray 1, Gray	Catalog # 890-00023
	AVITI Wash Tray 2, White	Catalog # 890-00027
	AVITI Waste Bottle	Catalog # 890-00024
Uline	[Optional] Heavy-Duty Funnel, 64 oz	Model # H-5216

CHAPTER 5

Consumable Prep

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Input Recommendations

The recommended input for sequencing is ≥ 1 nM library. The input library is normalized to 1 nM, denatured into single strands, and diluted to the target loading concentration. When starting with a 0.2–1 nM library, the library is denatured and diluted but not normalized. Libraries < 0.2 nM are not supported.

PhiX Control Library Spike-In

For most applications, Element recommends a spike-in of PhiX Control Library. The following spike-in percentages optimize the benefits of PhiX Control Library for specific experiments.

Table 7: Spike-in recommendations

Experiment	Spike-In (%)
QC and error rate reporting	> 2
Low-diversity libraries*	≥ 5
Low-complexity indexing (≥ 2 -plex)	> 2

* The first five cycles of Read 1 require high diversity.

Loading Concentration

The optimal loading concentration achieves a total polony count that ranges from ~800 million to 1 billion reads. The polony count increases as the loading concentration increases, which can compromise data quality. Low polony counts promote high data quality but lower the amount of data output.

The following loading concentrations target the total polony count range and provide a starting point for determining your optimal loading concentration. Some libraries require a loading concentration that is higher or lower than the indicated ranges. If you are sequencing pooled libraries, the pool must contain libraries with similar size distributions.

Table 8: Target loading concentrations

Library Size (bp)*	Adept Loading Concentration (pM)	Elevate Loading Concentration (pM)
Small (150–350)	5–7	5–9
Medium (350–600)	7–11	7–10
Large (≥ 600)	11–15	12–18

* Library size is the full length of the library, including the DNA insert, adapters, and primers.

Custom Primers

The AVITI System supports any combination of I1, I2, R1, and R2 custom primers to sequence Adept libraries. The custom primers must be HPLC-purified and prepared using the applicable method:

- **Spike-in**—Spike custom primers into the primer tubes provided in the AVITI Oligonucleotide Set or Adept Primer Set Cloudbreak.
- **Replacement**—Replace the primers in the cartridge with buffer tubes from the Adept Custom Oligonucleotide Buffer Set or Adept Custom Primer Set Cloudbreak and add custom primers.

Element oligonucleotides include sequencing primers that are only compatible with standard Nextera and TruSeq libraries. Adept libraries with sequencing primer binding sites that are not standard Nextera or TruSeq require custom primers.

Custom primers require special consideration and planning. To make sure a run that includes custom primers meets specifications, contact Element Technical Support early in experiment planning. Element Technical Support can also help determine whether your library requires custom primers and which preparation method applies.

Prepare the Cartridge

Preparing the sequencing cartridge adds Adept primer tubes as needed and thaws reagents. The subsequent dilution procedure includes the option to store a normalized library. If you intend to store, do not prepare the cartridge until you are ready to sequence. Prepare the cartridge within a day or two of sequencing.

CAUTION The cartridge contains light-sensitive reagents. **Protect the cartridge from light** until loading onto the instrument. Additionally, do not remove the cartridge lid.

Add Adept Primer Tubes

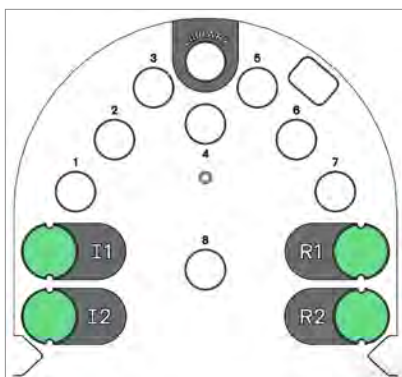
1. If you are sequencing Elevate libraries, skip the following steps and proceed to [Thaw the Cartridge on page 44](#).
—The sequencing cartridge contains Elevate primers, so only Adept libraries require primer replacement.—
2. Remove a cartridge and primer set from -25°C to -15°C storage. Reference the following table to determine the applicable primer set.

Primer Strategy	Primer Set
No custom primers	AVITI Oligonucleotide Set or Adept Primer Set Cloudbreak
Custom primers (spike-in method)	AVITI Oligonucleotide Set or Adept Primer Set Cloudbreak
Custom primers (replacement method)	Adept Custom Oligonucleotide Buffer Set or Adept Custom Primer Set Cloudbreak

—Subsequent procedures prepare custom primers and load them into the cartridge.—

3. Twist the primer tubes in wells I1, I2, R1, and R2 left to unlock.

Figure 14: Primer tube wells



4. Remove the primer tubes from the cartridge and discard per the SDS.
5. Insert the tubes from the primer set into the vacated wells. Match the abbreviation on the tube label to the well label.
—For example, insert Adept Custom Index 1 (**I1**) Buffer Cloudbreak into the **I1** well.—
6. Twist each tube right until it locks into place.

Thaw the Cartridge

Use a water bath (recommended) **or** refrigerator to thaw reagents in the cartridge. Steps to mix reagents and load diluted sequencing library into the cartridge occur during run setup.

Thaw Reagents in a Water Bath

1. If necessary, remove a cartridge from -25°C to -15°C storage.
2. Prepare a room-temperature water bath.
3. Place the cartridge in the water bath. Do not submerge.
4. Thaw the cartridge for the applicable duration:
 - » For a 2 x 75 cartridge, thaw for ~90 minutes. Place an object onto the cartridge to prevent floating and tilting.
 - » For a 2 x 150 cartridge, thaw for ~2.5 hours.
5. While the cartridge thaws, denature and dilute the library. See [Dilute Library and Custom Primers on page 45](#).
6. Inspect each well of the cartridge wells to make sure reagents are fully thawed.
7. If any ice remains, return the cartridge to the water bath until fully thawed.
8. Set aside the thawed cartridge at room temperature. If you are not sequencing immediately, keep the cartridge at 2°C to 8°C.

Thaw Reagents in a Refrigerator

1. If necessary, remove a cartridge from -25°C to -15°C storage.
2. Place the cartridge in a 2°C to 8°C refrigerator.
3. Thaw the cartridge for the applicable duration:
 - » For a 2 x 75 cartridge, thaw for ~8 hours.
 - » For a 2 x 150 cartridge, thaw for ~24 hours.
4. Inspect each well of the cartridge wells to make sure reagents are fully thawed.
5. If any ice remains, continue thawing:
 - a. Place the cartridge in a room-temperature water bath. Do not submerge.
 - b. Thaw the cartridge for the applicable duration:
 - For a 2 x 75 cartridge, thaw for ≤ 1 hour. Place an object onto the cartridge to prevent floating and tilting.
 - For a 2 x 150 cartridge, thaw for ≤ 20 minutes.
 - c. While the cartridge thaws, denature and dilute the library. See [Dilute Library and Custom Primers on page 45](#).
6. Set aside the thawed cartridge at room temperature. If you are not sequencing immediately, keep the cartridge at 2°C to 8°C.

Dilute Library and Custom Primers

The library dilution procedures prepare 1.4 ml diluted sequencing library at the target loading concentration with an optional spike-in. Custom primers are also diluted as applicable.

Prepare the Library

1. Gather the following consumables:
 - » 0.2 M Tris-HCl buffer, pH 7.0
 - » 1 N NaOH
 - » 2 ml DNA LoBind tubes (4–7)
 - » 10 mM Tris-HCl, pH 8.0 with 0.1 mM EDTA (low TE buffer)
 - » Nuclease-free water
2. Combine the following reagents to prepare 0.2 N NaOH. Use 0.2 N NaOH within the day and discard.

Reagent	Volume (μl)
1 N NaOH	20
Nuclease-free water	80
Total	100

3. Remove the following components from -25°C to -15°C storage and thaw on ice:
 - » Library Loading Buffer
 - » Experimental library
 - » [Optional] PhiX Control Library
4. Pulse vortex the thawed libraries and briefly centrifuge.
5. If the experimental library is ≥ 1 nM, normalize:
 - a. In a new DNA LoBind tube, use low TE buffer to dilute the library to 1 nM.
 - b. Proceed immediately or cap the tube, store the 1 nM library at -25°C to -15°, and sequence within the allotted time.

—Adept and Elevate v1 libraries require sequencing within 15 days of circularization.—

Denature the Library with NaOH

1. Calculate the loading concentration of each library, experimental and control, based on the target loading concentration and relative amount of each library:

$$\text{loading concentration in pM} = \text{target loading concentration in pM} * \text{library amount in \%}$$

—For example, if the target loading concentration is 9 pM with a 2% spike-in: the experimental library concentration is 8.82 pM (9 pM * 98%) and the control library concentration is 0.18 pM (9 pM * 2%).—



NOTE

The experimental and control library concentrations do not need to match.

2. Calculate the experimental library volume based on the calculated loading concentration and a 1.4 ml loading volume:

$$\text{library volume in } \mu\text{l} = (\text{library loading concentration in pM} * 1400 \mu\text{l}) / \text{library starting concentration in pM}$$

—Continuing the preceding example and assuming a 1 nM starting concentration, the library volume is 12.3 μl : $(8.82 \text{ pM} * 1400 \mu\text{l}) / 1000 \text{ pM}$.—

3. If you are adding a spike-in, calculate the control library volume based on the loading concentration and a 1.4 ml loading volume:

$$\text{control library volume in } \mu\text{l} = (\text{control library loading concentration in pM} * 1400 \mu\text{l}) / \text{control library concentration in pM}$$

—Continuing the preceding example and assuming a 1 nM PhiX Control Library, the control library volume is 0.25 μl : $(0.18 \text{ pM} * 1400 \mu\text{l}) / 1000 \text{ pM}$.—

4. If step 3 calculated a volume < 1 μl , dilute PhiX Control Library in low TE buffer to use a volume $\geq 1 \mu\text{l}$ for accurate pipetting.
5. Record the total volume of diluted sequencing library (experimental and control) in μl .
—This procedure uses equal volumes of library, 0.2 N NaOH, and 0.2 M Tris-HCl buffer, pH 7.0. Therefore, the volume recorded at this step is used in two subsequent steps.—
6. Combine the library volumes calculated in steps 2 and 3 in a new DNA LoBind tube.
7. Add an equal volume of freshly prepared 0.2 N NaOH.
8. Vortex the tube to mix and briefly centrifuge.
9. Incubate the tube at room temperature for 5 minutes to denature the library.
10. Vortex the tube to mix and briefly centrifuge.
11. Add 0.2 M Tris-HCl buffer, pH 7.0 at an equal volume of 0.2 N NaOH to neutralize the reaction.
12. Vortex the tube to mix and briefly centrifuge.
—The library is denatured, neutralized, and at 1/3 the input concentration in 3x input volume.—
13. Add a sufficient volume of Library Loading Buffer to reach a total volume of 1.4 ml:

$$\text{buffer volume in } \mu\text{l} = 1400 \mu\text{l} - 3 * \text{library volume in } \mu\text{l}$$

14. Vortex the tube to mix and briefly centrifuge.
15. Place the diluted sequencing library on ice. Use within the day.

Prepare Custom Primers

1. If you are not using custom primers, skip the following steps and proceed to [Set Up a Run on page 48](#).
2. In a new DNA LoBind tube, prepare each applicable custom primer using low TE buffer:

Custom Primer	Volume (μl)	Concentration (μM)
Index 1	19	100
Index 2	19	100
Read 1	32.4	100
Read 2	19	100

3. Set aside the 100 μM custom primers on ice. Use within the day.

CHAPTER 6

Sequencing

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Set Up a Run

Setting up a run defines the run parameters and loads sequencing consumables. For help discarding run setup, recovering from errors, and other troubleshooting information, see [Troubleshooting on page 82](#).

**NOTE**

Before priming, you can discard run setup and save the cartridge.

Initiate a Sequencing Run

1. Gather the following materials:
 - » Buffer bottle
 - » Cartridge
 - » Sequencing basket
 - » Towel or wipe
 - » Used flow cell—A used flow cell might already be in the instrument.—
2. If applicable, stage run manifests for import:
 - a. Save a run manifest to a USB drive or SMB within the path specified for the storage connection.
 - b. Repeat step [a](#) as needed to stage a second run manifest for a dual start run.
 - c. If you saved run manifests to a USB drive, connect the USB drive to a port on the side or back of the instrument.
3. On the Home screen, select **New Run**.
4. If AVITI OS prompts that the flow cell is missing, load a **used** flow cell:
 - a. Select **Open Nest**.
 - b. Place the used flow cell onto the nest and close the lid.
 - c. Select **Close Nest**.
5. Select which side to sequence on:
 - » **Side A**—Set up a run on side A.
 - » **Both**—Set up runs on sides A and B.
 - » **Side B**—Set up a run on side B.
6. Select **Sequence**, and then select **Next** to proceed to the Prepare Reagents screen.

Inspect and Mix Reagents

1. Inspect each cartridge well to make sure reagents are fully thawed.
2. Make sure the cartridge contains the appropriate primers.
3. Make sure the tubes in the I1, I2, R1, and R2 wells are secure. If necessary, twist each tube to the left.
4. Gently invert the cartridge **10 times** to mix reagents.

**CAUTION**

Inadequately mixed reagents can cause run failure.

5. Tap the cartridge base on the benchtop to remove any large droplets from the tube tops.

6. Inspect the small tubes to make sure reagents are settled at the bottom.
7. Place the cartridge into a clean sequencing basket and lock the clips. Wipe any excess moisture.

Add Custom Primers to the Cartridge

1. If you are not using custom primers, skip the following steps and proceed to [Add Library to the Cartridge](#).
2. Using a new 1 ml pipette tip, pierce the center of the applicable I1, I2, R1, and R2 wells to create one hole. Push the foil to the edges.
3. Discard the pipette tip.
4. Add the applicable volume of 100 μ M custom primer to each pierced well.

Custom Primer	Volume (μ l)	Well
Index 1	19	I1
Index 2	19	I2
Read 1	32.4	R1
Read 2	19	R2

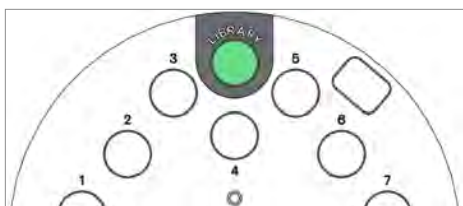
—The final concentration of each custom primer is 1 μ M.—

5. Pipette the content of each tube 15 times to mix. Avoid losing existing primer volume.

Add Library to the Cartridge

1. Using a new 1 ml pipette tip, pierce the center of the Library well to create one hole. Push the foil to the edges.

Figure 15: Library well location



2. Discard the pipette tip.
3. Briefly centrifuge the diluted sequencing library to remove bubbles and foam from the tube lid.
4. Transfer the entire volume of diluted sequencing library to the Library well, dispensing along the well wall.
 - » Avoid aspirating any foam or dispensing air.
 - » Do not allow the library to contact the foil.
 - » Make sure the tube contains ≥ 1.3 ml diluted sequencing library.
5. Inspect the library through the window at the front of the basket.
 - » Make sure the library is free of foam and that bubbles are minimal.
 - » If an air gap appears below the surface, use a new pipette tip to remove it.
6. Twist each shipping lock left to unlock.
7. Remove both shipping locks from the cartridge lid.

8. [Optional] Set aside one shipping lock for use during reagent disposal.

Select a Library Prep Workflow

1. Select a library prep workflow:
 - » **Adept**—Sequence libraries prepared with the Adept Workflow.
 - » **Elevate**—Sequence libraries prepared with Elevate indexes and adapters.
2. If you selected Adept, select the **Swap primer tubes** checkbox to confirm that the I1, I2, R1, and R2 wells contain Adept primers or custom primers.
3. Select the **Invert cartridge** checkbox to confirm that reagents are mixed.
4. Select the **Load library pool** checkbox to confirm that the Library well contains diluted sequencing library.
5. Select **Next** to proceed to the Load Reagents screen.

Load Reagents and Buffer

1. Open the reagent bay door.
2. Remove any materials from the reagent bay and set aside.
3. Slide the basket containing the thawed cartridge into the reagent bay until it stops.
4. Support the buffer bottle with both hands and slide it into the reagent bay until it stops.
5. Close the reagent bay door.
6. Select **Next** to proceed to the Run Side A or Run Side B screen.

Define Run Parameters

1. In the Run Name field, enter a unique name to identify the run.
 - The field accepts 1–64 alphanumeric characters, hyphens (-), and underscores (_).
2. If applicable, import the run manifest:
 - a. Select **Browse**, and then browse to the run manifest for the current run.
 - b. Select the run manifest, and then select **Download**.
3. Select a Scan Area option:

Option	Approximate Output*
Full Scan (default)	1 billion reads
3/4	750 million reads
1/2	500 million reads
1/4	250 million reads
1/8	125 million reads

* Read count based on Element control library sequencing. Actual read count might differ based on factors such as library type and preparation.

4. In the Storage list, select a storage location:

- » To output run data to the default storage location, leave the default selection.
- » To override the default storage location for the current run, select a storage connection.
- 5. [Optional] In the Description field, enter a description that represents the run.
—The field accepts ≤ 500 alphanumeric characters, hyphens, underscores, spaces, and periods (.).—
- 6. In the Cycles fields, enter the number of cycles to perform in each read. Add one cycle to the desired number of Read 1 and Read 2 cycles for bioinformatics purposes.

Workflow	Kit	Valid Values				Default Values			
		Index 1	Index 2	Read 1	Read 2	Index 1	Index 2	Read 1	Read 2
Adept v1, v1.1	2 x 75	0–75	0–75	5–184	0–179	Blank	Blank	76	76
	2 x 150	0–75	0–75	5–334	0–329	Blank	Blank	151	151
Adept with Cloudbreak	2 x 75	0–32	0–32	5–184	0–179	Blank	Blank	76	76
	2 x 150	0–32	0–32	5–334	0–329	Blank	Blank	151	151
Elevate v1	2 x 75	0–75	0–75	5–184	0–179	12	9	76	76
	2 x 150	0–75	0–75	5–334	0–329	12	9	151	151
Elevate with Cloudbreak	2 x 75	4–32	0–32	5–180	0–175	12	9	76	76
	2 x 150	4–32	0–32	5–330	0–325	12	9	151	151

—For example, enter 151 in the Read 1 field to perform 150 cycles in Read 1. Entering zero cycles skips the read.—

- 7. Select **Next** to proceed to the Run Side B or Empty Waste screen.
- 8. If applicable, repeat steps 1–7 to set up a dual start run.

Empty Waste and Prime Reagents

1. Open the waste bay door.
2. Unscrew the transport cap from the cap holder above the waste bay.
3. Remove the waste bottle from the waste bay and close the transport cap.
4. [Optional] Insert a funnel into a waste receptacle. Make sure the funnel is secure.
5. Open the transport cap and the vent cap.
6. Support the waste bottle with both hands and empty the waste:
 - a. Position the bottle over the funnel or waste receptacle.
 - If you inserted a funnel, align the handle to the inner edge of the funnel.
 - If you did not insert a funnel, center the handle over the waste receptacle.
 - b. Tip the bottle forward and drain. Invert the bottle and shake to expel all droplets.
 - c. If necessary, wipe liquid off the bottle.
7. Close the vent cap and return the empty waste bottle to the waste bay.
8. Screw the transport cap onto the cap holder and close the waste bay door.
9. Select **Next** to proceed to the Priming screen and **automatically** start priming.
—Priming pierces the cartridge seals, so the cartridge cannot be used for another run.—
10. During priming, which takes ~14 minutes, bring a new flow cell to room temperature:

- a. Remove a flow cell pouch from 2°C to 8°C storage. **Do not open the pouch.**
 - b. Set aside the pouch for ≥ 5 minutes.
11. When priming is complete, select **Next** to proceed to the Load Flow Cell screen.
- AVITI OS moves the nest forward and opens the nest bay door. A brief delay is normal.—

Load the Flow Cell

1. Make sure the nest status light is blue.
2. Press the button to the left of the nest to open the lid.
3. Remove the used flow cell from the nest.
4. Discard the used flow cell or store at room temperature to use for priming and washes.
5. Unpackage the new flow cell. Handle the flow cell by the gripper only.

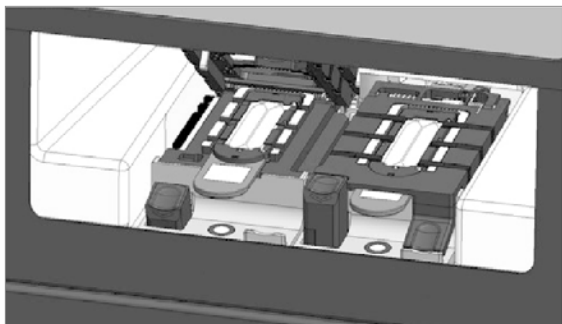


CAUTION

Touching the glass can introduce debris, smudges, and scratches, compromising data quality.

6. Face the label up and place the flow cell over the three registration pins on the nest.

Figure 16: Loaded flow cells



7. Lower the tab on the right side of the lid until the lid snaps into place.
—The nest status light turns green.—
8. Select **Close Nest** to close the nest bay door and retract the stage.
9. Select **Next** to proceed to the Run Summary screen.

Review and Start the Run

1. Review the run parameters:

Parameter	Description
Workflow	The workflow that prepared the libraries
Kit Size	The supported read length for the sequencing cartridge
Chemistry	The version of the sequencing cartridge and flow cell
Storage	The location where sequencing data are output
Scan Area	The area of the flow cell that the system scans
Cycles	The number of cycles in each read
Description	An optional description of the run

2. Review the flow cell, cartridge, and buffer bottle information:

Field	Description
Lot Number	The number assigned to the batch the consumable was manufactured with
Expires on	The year, month, and date that the consumable expires
Serial Number	The unique identifier for the consumable or all zeros indicating an unscanned barcode
Part Number	The Element-assigned identifier for the consumable

—A warning alerts you to expired consumables. Although not supported, AVITI OS allows the run to proceed.—

3. Select **Run** to start sequencing.
 4. [Optional] If you imported run manifests from a USB drive, disconnect the USB drive:
 - a. In the taskbar, select **USB Drive**, and then select **Eject**.
 - b. Detach the USB drive from the instrument.
 5. Process the materials removed from the reagent bay:
 - » If you removed a used cartridge and buffer bottle, follow the instructions in [Discard the Cartridge and Bottle on page 55](#).
 - » If you removed a wash tray, follow the guidelines in [Wash Tray Maintenance on page 59](#).
- Residual wash solution in the wash tray is normal.—

Monitor Run Metrics

1. If necessary, select **Details** to open run details.
2. Monitor run metrics as they appear onscreen:
 - a. After Index 1, cycle 5 for the Elevate Workflow, review Reads and Yield.

- b. Within 10 minutes of the index cycles completing, select the **Indexing Stats** tab to review Assigned, Perfect Match, and Samples with Low Representation.
- c. After Read 1, cycle 1 for the Adept Workflow, review Reads and Yield.
- d. After Read 1, cycle 10, review Q30 on the Home screen. Select the **% Q30** tab to see cycle-by-cycle details.
- e. After Read 1, cycle 25, select the **PhiX Error** tab to see the error rates for a run that includes a spike-in.

—All cycles are approximate and all metrics are estimates. Bases2Fastq generates the final metrics.—

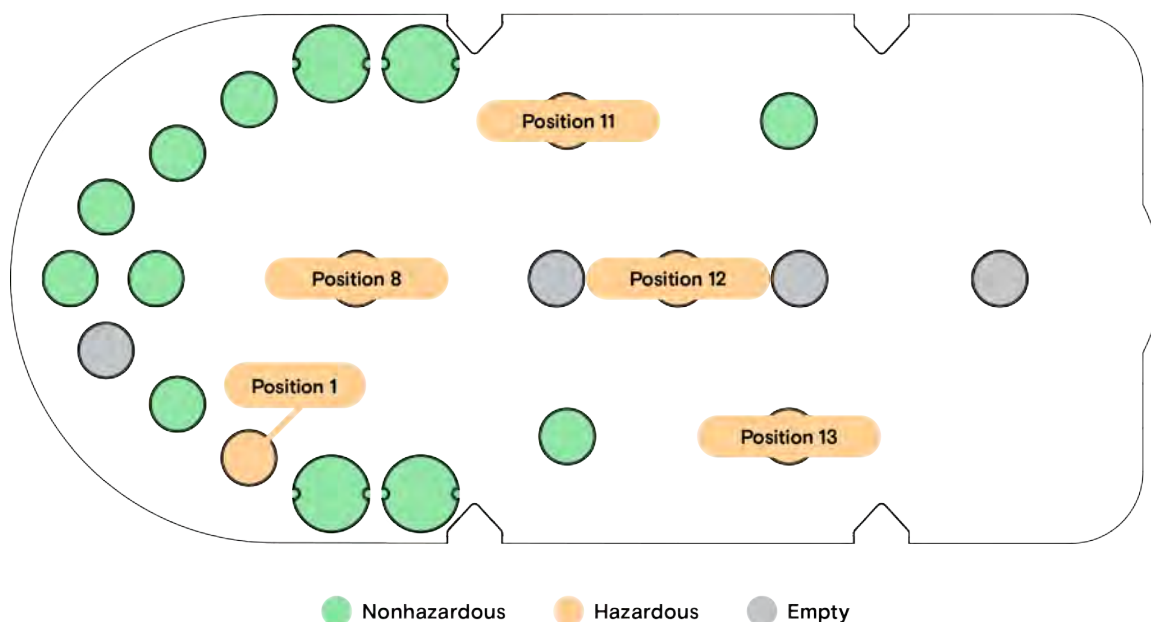
- 3. Continue monitoring the run as AVITI OS refreshes the metrics.
 - » Each cycle refreshes the Q30 scores, error rates, and index metrics.
 - » AVITI OS refreshes the yield and reads metrics after cycle 15 of Read 2:
 - If Read 2 contains no cycles, the refresh occurs after cycle 15 of Read 1.
 - If Read 1 or Read 2 contain < 15 cycles, the refresh occurs when the last cycle of the read starts.
- 4. If a run needs to be stopped, see [Stop an Active Run on page 85](#).
- 5. When the run is complete, leave all materials in the instrument.
 - » To return to the Details view, select **History**.
 - » To access run data, go to your storage location.

Discard the Cartridge and Bottle

The cartridge and buffer bottle contain reagents with region-specific disposal requirements, which are described in the SDS at go.elembio.link/sds. The amount of reagent remaining in each well of the cartridge after a run depends on how many cycles the run performed.

The following figure identifies the hazardous reagents in a Cloudbreak cartridge. The reagent position numbers in the figure align with the position numbers in the SDS.

Figure 17: Cloudbreak cartridge map (part # 820-00006, 820-00007)

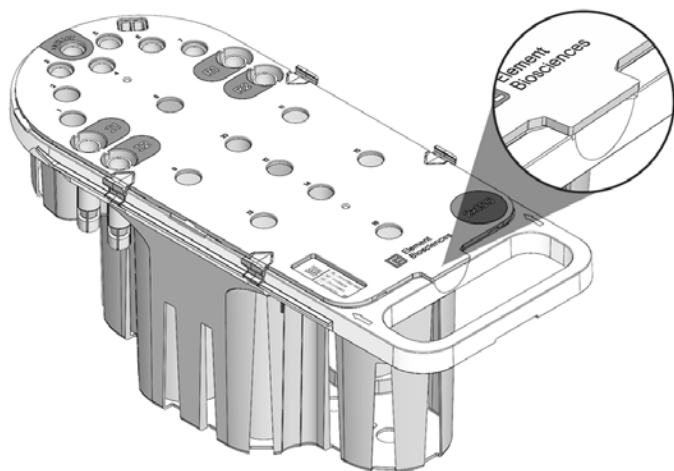


Dispose of Version 1 Reagents

1. Unlock the four clips on top of the basket.
2. Remove the cartridge from the basket.
—The tabs and divots at each end facilitate removal.—
3. Discard the cartridge and buffer bottle per the SDS.
—The volume remaining in each depends on the number of cycles performed.—
4. Rinse the basket with nuclease-free water and dry upside down.

Dispose of Cloudbreak Reagents

1. Keep the cartridge in the basket with the clips locked.
2. Grip the lid tab and **quickly and forcefully** pull off the lid. Expect resistance.

Figure 18: Lid tab location

3. Remove the wells marked hazardous from the cartridge.
—The volume remaining in each well depends on the number of cycles performed.—
4. Using the nub on the bottom of a shipping lock or similar tool, enlarge the hole in each foil seal to form a triangle.

Figure 19: Triangular hole in a foil seal

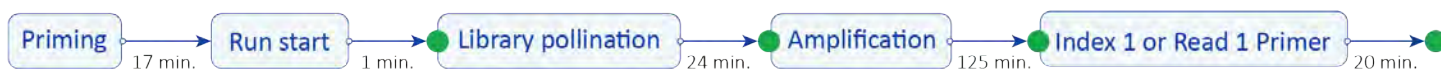
—The triangular shape facilitates pouring.—

5. Empty each well into hazardous waste or other appropriate container per the SDS.
6. Unlock the clips and remove the cartridge from the basket.
7. Remove the remaining wells from the cartridge.
8. Enlarge the hole in each foil seal as described in step 4.
9. Empty each well into the appropriate container per the SDS.
10. Discard the cartridge and buffer bottle per the SDS.
11. Rinse the basket with nuclease-free water and dry upside down.

Initiate Flexible Start

1. On the Home screen, select **New Run**.
2. When prompted to request flexible start and pause the active run, select **New Run**. Reference the green dots in the following figure to determine optimal pause points with minimal downtime.

Pollination and Amplification



Cycling



Paired-End Turn



Index Priming



—Pausing typically takes several minutes but can take as long as ~2 hours. Durations for the depicted run stages are approximate.—

3. Wait for the run to pause. To cancel flexible start while waiting, select **Cancel Request**.
4. When the run pauses, proceed through setting up and starting the second run. To cancel setup:
 - a. Select **Back** to return to the Home screen, and then select **Resume**.
 - b. When prompted to confirm that you want to resume the active run, select **Resume**.

—After you start or cancel the second run, AVITI OS resumes the active run.—

CHAPTER 7

Maintenance

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Maintenance Schedule

Element recommends the following maintenance schedule to keep the AVITI System in optimal condition and help maintain performance. Every 7 days, AVITI OS warns you that a maintenance wash is due. AVITI OS repeats the warning every 2 days until you perform the wash.

Procedure	Frequency	Purpose
Power cycle	Weekly	Reinitializes the system and resets the instrument computer, which helps maintain instrument performance.
Maintenance wash*	Weekly	Cleans the outside of the sippers and prevents microbial growth and particulate debris from accumulating in the fluidic system.
Standby wash*	Preparing for an idle period of ≥ 7 days	Prepares one or both sides for an idle period of ≥ 7 days.
Air filter replacement	Every 6–12 months	Ensures proper cooling and continuous operation. The optimum frequency depends on lab cleanliness.

* For information on a third wash type, recovery, see [Stop an Active Run on page 85](#).

Exterior Cleaning

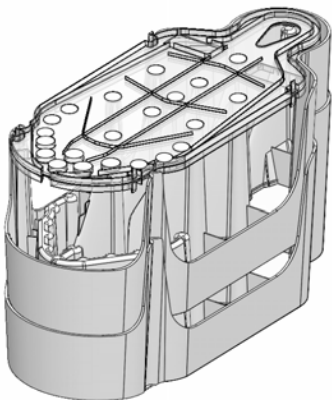
Clean the instrument exterior as needed by wiping the shells with a damp microfiber cloth and using Simple Green to remove oils and fingerprints. Avoid harsh chemicals, cleaners, and abrasives.

Wash Tray Maintenance

The following guidelines for wash tray maintenance keep the wash trays in good condition, maximizing time between replacements and preventing cross-contamination.

- When transporting a wash tray, optionally cover the wash tray with a lid to help prevent spills. Otherwise, a lid is not required.
 - » Keep the lids that come with the gray wash trays with the gray wash trays.
 - » Keep the lids that come with the white wash trays with the white wash trays.
- After each use, discard residual wash solution, rinse the wash tray with nuclease-free water, and air-dry upside down. Rinse the lid with nuclease-free water and air-dry.
- Store clean, dry wash trays upside down with the lids on top of the inverted trays. Stack up to two wash trays with two lids.

Figure 20: Stored wash trays



Warranties and Services

The purchase of an Element AVITI System includes a standard one-year warranty, installation procedures, operational procedures, a performance run, and applications training. For an additional cost, Element offers supplemental procedures, preventative maintenance services, and extended warranties.



NOTE

Element is both the manufacturer of the AVITI System and the only authorized service provider.

Power Cycle the System

A power cycle resets the instrument computer, safely shutting down and restarting the system to maintain performance or recover from a problem. Turning off the system without a proper power cycle is reserved for emergencies.

1. Select the user menu, and then select **Shut Down**.
2. When prompted, select **Shut Down** again to shut down the instrument computer.
3. Wait a few seconds for the screen to go blank.
4. On the IO panel on the back of the instrument, press the power toggle switch to turn off the instrument.

Figure 21: Power toggle switch in the on position



5. Wait **10 seconds** to make sure the system fully shuts down.
6. On the IO panel, press the power toggle switch to turn on the instrument.
—The system initializes and displays the Home screen.—
7. If a USB drive is connected to the instrument, reconnect it:
 - a. In the taskbar, select **USB Drive**, and then select **Eject**.
 - b. Detach the USB drive from the instrument.
 - c. Reconnect the USB drive to the instrument.
—Reconnecting the USB drive allows AVITI OS to detect it after a power cycle.—

Perform a Maintenance Wash

The maintenance wash is a two-part wash that takes a total of ~1.5 hours. Wash 1 cleans the system, removing residual library and carryover. Wash 2 rinses the system, removing residual Wash 1 solution and preparing for the next run. Each wash requires specific volumes of freshly prepared wash solutions.

Prepare Wash Solutions

1. Gather the following materials:
 - » 2 L bottles (2)
 - » 4.00–4.99% sodium hypochlorite
 - » Gray wash tray
 - » Nuclease-free water
 - » Pipette controller
 - » Serological pipettes (2)
 - » Tween 20
 - » Used flow cell
 - » White wash tray

—A used flow cell might already be in the instrument.—
2. Add 1.5 L nuclease-free water to a new 2 L bottle.
3. Attach a new serological pipette to a pipette controller.
4. Add 37.5 ml 4.00–4.99% sodium hypochlorite to the bottle to prepare 1.54 L ~0.12% sodium hypochlorite.
5. Label the bottle **Wash 1 Solution**.
6. Cap the bottle and invert several times to mix.
7. Set aside Wash 1 Solution at room temperature. Use within the day or discard.
8. Add 1.5 L nuclease-free water to a new 2 L bottle.
9. Attach a new serological pipette to the pipette controller.
10. Add 4.5 ml Tween 20 to the bottle to prepare 1.5 L 0.3% Tween 20.
11. Label the bottle **Wash 2 Solution**.
12. Cap the bottle and invert several times to mix.
13. Set aside Wash 2 Solution at room temperature.

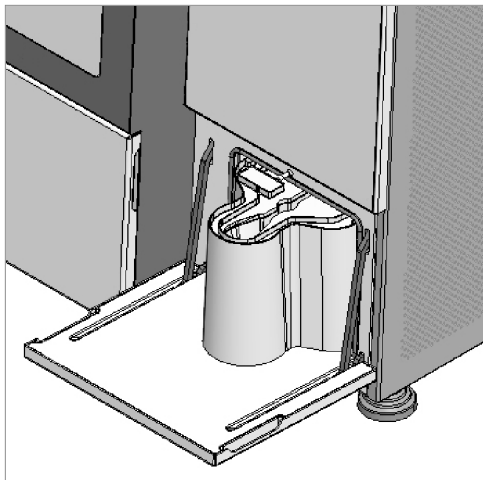
Initiate a Maintenance Wash

1. On the Home screen, select **New Run**.
2. If AVITI OS prompts that the flow cell is missing, load a *used* flow cell:
 - a. Select **Open Nest**.
 - b. Place the used flow cell onto the nest and close the lid.
 - c. Select **Close Nest**.
3. Select which side to wash:
 - » **Side A**—Set up a maintenance wash on side A.

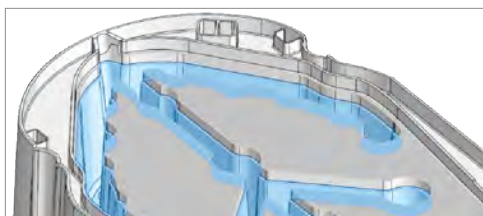
- » **Both**—Set up maintenance washes on sides A and B.
 - » **Side B**—Set up a maintenance wash on side B.
4. Select **Wash**, and then select **Maintenance**.
 5. Select **Next** to proceed to the Load Wash 1 screen.

Load Wash 1 Solution

1. Open the reagent bay door.
2. Remove any materials from the reagent bay and set aside.
3. Place a clean, uncovered gray wash tray onto the open door.
4. Slide ~2/3 of the wash tray into the reagent bay, so the barcode edge is about flush with the entrance.



5. Add 590 ml freshly prepared Wash 1 Solution to the fill area, filling the wash tray to slightly above the lower fill line.



6. Slide the wash tray all the way into the reagent bay until it stops and close the reagent bay door.
7. Select **Next** to proceed to the Empty Waste screen.

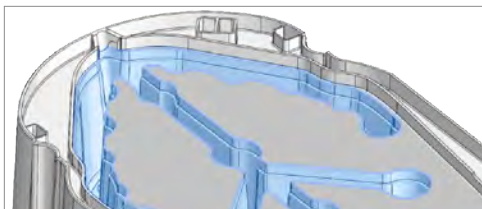
Empty Waste and Run Wash 1

1. Open the waste bay door.
2. Unscrew the transport cap from the cap holder above the waste bay.
3. Remove the waste bottle from the waste bay and close the transport cap.
4. [Optional] Insert a funnel into a waste receptacle. Make sure the funnel is secure.
5. Open the transport cap and the vent cap.
6. Support the waste bottle with both hands and empty the waste:

- a. Position the bottle over the funnel or waste receptacle.
 - If you inserted a funnel, align the handle to the inner edge of the funnel.
 - If you did not insert a funnel, center the handle over the waste receptacle.
 - b. Tip the bottle forward and drain. Invert the bottle and shake to expel all droplets.
 - c. If necessary, wipe liquid off the bottle.
7. Close the vent cap and return the empty waste bottle to the waste bay.
 8. Screw the transport cap onto the cap holder and close the waste bay door.
 9. Select **Next** to open the Run Wash 1 screen and automatically start the wash, which takes ~34 minutes.
 10. During the wash, process the materials removed from the reagent bay:
 - » If you removed a used buffer bottle and sequencing basket, follow the instructions in [Discard the Cartridge and Bottle on page 55](#).
 - » If you removed a wash tray, follow the guidelines in [Wash Tray Maintenance on page 59](#).
 11. When the wash is complete, select **Next** to proceed to the Load Wash 2 screen.

Load Wash 2 Solution

1. Open the reagent bay door.
2. Remove the gray wash tray from the reagent bay and set aside.
—Residual wash solution in the wash tray is normal.—
3. Place a clean, uncovered white wash tray onto the open door.
4. Slide ~2/3 of the wash tray into the reagent bay, so the barcode edge is about flush with the entrance.
5. Add 660 ml freshly prepared Wash 2 Solution to the fill area, filling the wash tray to slightly above the upper fill line.



6. Slide the wash tray all the way into the reagent bay until it stops and close the reagent bay door.
7. [Optional] Store leftover Wash 2 Solution at 2°C to 8°C for ≤ 2 weeks.

Run Wash 2

1. Select **Next** to open the Run Wash 2 screen and automatically start the wash, which takes ~52 minutes.
2. When the wash is complete, select **Done** to return to the Home screen.
3. Leave all materials in the instrument.
4. Process the gray wash tray from the first wash per [Wash Tray Maintenance on page 59](#).

Perform a Standby Wash

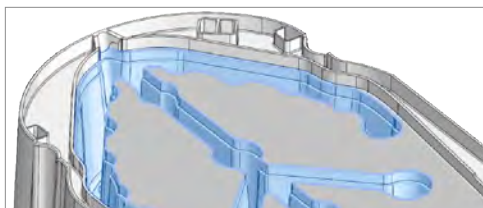
A standby wash takes ~52 minutes and flushes nuclease-free water through the fluidic system, removing any residual Tween 20. When complete, the washed side is idle. Performing a maintenance wash on the idle side ends the idle period and enables sequencing.

Initiate a Standby Wash

1. Gather the following materials:
 - » Nuclease-free water
 - » Used flow cell
 - » White wash tray
 —A used flow cell might already be in the instrument.—
2. On the Home screen, select **New Run**.
3. If AVITI OS prompts that the flow cell is missing, load a *used* flow cell:
 - a. Select **Open Nest**.
 - b. Place the used flow cell onto the nest and close the lid.
 - c. Select **Close Nest**.
4. Select which side to wash:
 - » **Side A**—Set up a standby wash on side A.
 - » **Both**—Set up standby washes on sides A and B.
 - » **Side B**—Set up a standby wash on side B.
5. Select **Wash**, and then select **Standby**.
6. Select **Next** to proceed to the Load Water screen.

Load Nuclease-Free Water

1. Open the reagent bay door.
2. Remove any materials from the reagent bay and set aside.
3. Place a clean, uncovered white wash tray onto the open door.
4. Slide ~2/3 of the wash tray into the reagent bay, so the barcode edge is about flush with the entrance.
5. Add 660 ml nuclease-free water to the fill area, filling the wash tray to slightly above the upper fill line.



6. Slide the wash tray all the way into the reagent bay until it stops.
7. Close the reagent bay door.
8. Select **Next** to proceed to the Empty Waste screen.

Empty Waste and Run the Standby Wash

1. Open the waste bay door.
2. Unscrew the transport cap from the cap holder above the waste bay.
3. Remove the waste bottle from the waste bay and close the transport cap.
4. [Optional] Insert a funnel into a waste receptacle. Make sure the funnel is secure.
5. Open the transport cap and the vent cap.
6. Support the waste bottle with both hands and empty the waste:
 - a. Position the bottle over the funnel or waste receptacle.
 - If you inserted a funnel, align the handle to the inner edge of the funnel.
 - If you did not insert a funnel, center the handle over the waste receptacle.
 - b. Tip the bottle forward and drain. Invert the bottle and shake to expel all droplets.
 - c. If necessary, wipe liquid off the bottle.
7. Close the vent cap and return the empty waste bottle to the waste bay.
8. Screw the transport cap onto the cap holder and close the waste bay door.
9. Select **Next** to open the Run Water screen and automatically start the wash.
10. When the wash is complete, select **Next** to proceed to the Remove Tray screen.

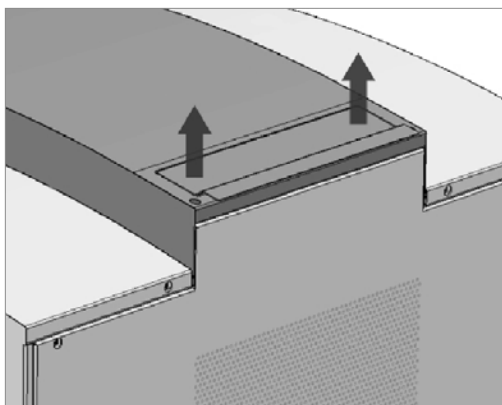
Unload the Wash Tray

1. When prompted, open the reagent bay door and remove the wash tray.
—Residual water in the wash tray is normal.—
2. Close the reagent bay door.
3. Select **Done** to proceed to the Home screen.
4. Leave the flow cell in the nest.
5. Process the materials removed from the reagent bay:
 - » If you removed a used buffer bottle and sequencing basket, follow the instructions in [Discard the Cartridge and Bottle on page 55](#).
 - » If you removed a wash tray, follow the guidelines in [Wash Tray Maintenance on page 59](#).

Replace the Air Filter

Replacing the air filter ensures proper cooling and continuous operation of the system.

1. If the instrument is sequencing or washing, wait for the run or wash to complete.
2. Select the user menu, and then select **Shut Down**.
3. When prompted, select **Shut Down** again to shut down the instrument computer.
4. Wait a few seconds for the screen to go blank.
5. On the IO panel on the back of the instrument, press the power toggle switch to turn off the instrument.
6. Using the flange toward the back of the instrument, lift the air filter tray out of the top.



7. Remove the air filter from the tray and discard.
—The filter might be loose in the tray, which is normal.—
8. Place the tray on a table or benchtop.
9. With the small arrow on the side of the filter pointing up, place the filter into the tray.
10. Lower the tray into the instrument. Use the pins to align the tray to the rails and guide entry.
11. On the IO panel, press the power toggle switch to turn on the instrument.
—The system initializes and displays the Home screen.—

CHAPTER 8

System Configuration

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System Connections

The AVITI System uses a combination of network, internet, and storage connections to operate. Each system requires a network connection and at least one storage connection. Cloud storage connections, telemetry, remote software updates, and remote support require an internet connection.

Mode	Network Connection	Internet Connection	Storage Connection
Online	Internet	DHCP or static	Cloud or local
	Local	DHCP or static	Local
Offline	Local	None	Local

System Modes

The system mode determines connection options and settings for exporting log files, password protection, and software updates:

- Online mode connects the system to the internet, which streamlines operations.
- Online local authentication mode operates in online mode but includes local authentication, which avoids network requirements to allowlist Auth0 IP addresses. Only an Element representative can enable this mode.
- Offline mode operates the system without an internet connection. Only an Element representative can enable offline mode.

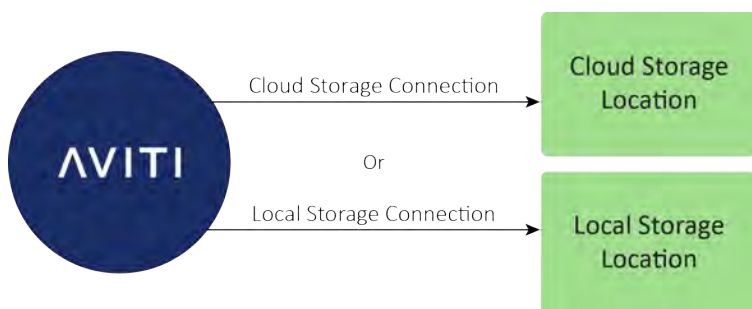
Storage Connections

A storage connection establishes an off-instrument location that AVITI OS transfers files to. Each run delivers bases files and other run outputs to the default storage location unless you specify a different location during run setup.

AVITI OS supports cloud and local storage connections:

- A cloud storage connection transfers files to a storage location in the cloud.
- A local storage connection transfers files to a storage location on a local network or USB drive.

Figure 22: Cloud versus local storage



Storage Connection Requirements

Adding a storage connection requires permissions, network information, and account information that your IT administrator can provide. A cloud storage connection requires an access key and a secret access key, which you can import from a CSV file saved to a USB drive or enter manually.

For comprehensive storage requirements, see the *Element AVITI System Site Prep Guide (MA-00007)*.

Supported Storage Connections

Cloud storage connections include Amazon Web Services (AWS) and Google Cloud Storage (GCS), which is part of the Google suite of cloud computing services. For local storage, AVITI OS supports Server Messenger Block (SMB) and USB.

The storage location for a cloud storage connection is a bucket. A connected bucket is available to all systems. Local storage is exclusive to the system.

Cloud Storage

Cloud Storage Connection	Description
AWS	<ul style="list-style-type: none">• Connects the system to an Amazon Simple Storage Service (S3) bucket.• Bases data transfer on secret key authentication through AWS Identity and Access Management (IAM).
GCS	<ul style="list-style-type: none">• Connects the system to a Cloud Storage bucket.• Bases data transfer on secret key authentication through a keyed-hash message authentication code (HMAC).

Local Storage

Local Storage Connection	Description
SMB	<ul style="list-style-type: none">• Connects the system to the server running SMB via a path to a folder.• Uses the SMB protocol based on service user authentication to transfer data.• Enables import of a run manifest from an SMB storage location during run setup.• Supports automatic export of log files from offline systems.• Supports Kerberos or NTLMv2 authentication.
USB	<ul style="list-style-type: none">• Transfers data and log files to a USB drive connected to the instrument.• Supports automatic and manual export of log files from offline systems.• Supports USB-A 3.0 or newer versions and FAT32 or exFAT formats.• Must store a minimum of 1.6 TB of data, which is sufficient for two 2 x 150 runs with indexing.

Configure General Settings

General settings include the instrument name setting and read-only settings that control the instrument profile. For offline systems, general settings also include features to export log files. For instructions, see [Exporting Log Files on page 79](#).

Name the Instrument

1. On the taskbar, select **Settings**.
2. Select the **General** tab, and then select **Edit**.
3. Enter a preferred name consisting of 1–20 alphanumeric characters, hyphens (-), and underscores (_) to identify the instrument.
—The default name is the serial number, field-programmable gate array (FPGA) ID, or Unnamed Instrument.—
4. Select **Save** to apply the name.

Review Read-Only Settings

1. On the taskbar, select **Settings**.
2. Select the **General** tab, and then select **Edit**.
3. Review the following read-only settings. To change a setting, contact Element Technical Support.

Setting	Default	Description
Telemetry	Enabled	Sends instrument health data to Element
High Elevation	Disabled	Calibrates the system to operate at a high elevation
Offline Mode	Disabled	Prevents an internet connection

—Disabling telemetry affects the instrument warranty.—

Connect to the Network

Network settings connect the system to your network via Dynamic Host Configuration Protocol (DHCP) or static IP address. When the system is connected to an Ethernet port, AVITI OS automatically connects to a DHCP server and autopopulates the network settings. Alternatively, you can assign a static IP address and manually configure the network settings.

Select a DHCP Server

1. On the taskbar, select **Settings**.
2. Select the **Network** tab.
3. In the drop-down list, select **Automatic (DHCP)**.
—AVITI OS assigns a dynamic IP address and all other network settings.—

Assign a Static IP Address

1. On the taskbar, select **Settings**.
2. Select the **Network** tab.
3. In the drop-down list, select **Manual**.
—AVITI OS assigns a unique and permanent IP address.—
4. Select **Edit**, and then configure the following network settings.

Setting	Example	Description
IP Address	11.2.34.178	The destination IP address
Gateway	11.2.34.177	The IP address of the gateway computer that manages network communications
Subnet Mask	11.2.34.176	The subnet mask that separates the IP address into host and network addresses
Name Server IP(s)	ngs-1.yourlab.com	The names of up to four Domain Name System (DNS) servers that provide IP addresses

—Two additional network settings, Host name and Mandatory Access Control (MAC) address, are read-only.—

5. Select **Save** to apply the settings and connect to the network.

Add Storage Connections

The Storage tab lists storage connections added to the system, including available storage for each local storage connection. An Element representative adds the first storage connection at installation. After installation, you can add an unlimited number of additional storage connections.

Add an AWS Storage Connection

1. If you are importing the access key and secret access key, prepare the CSV file:
 - a. Save the CSV file to a compatible USB drive.
 - b. Connect the USB drive to a USB port on the side or back of the instrument.
2. On the taskbar, select **Settings**.
3. Select the **Storage** tab.
4. Select **Add Storage**, and then select **AWS** as the storage provider.
5. In the Name field, enter a preferred name for the storage connection.
6. Complete the following fields to configure an Amazon S3 bucket as the storage location.

Field	Instruction
Bucket Name	Enter the bucket name, such as NGS-AWS-123.
S3 Prefix	Enter the prefix for the bucket, such as Bucketname/Bucket/January.
Region	Select the region associated with the bucket.

—The Bucket Name and Region fields are required.—

7. Complete the Access Key and Secret Access Key fields using either method:
 - » To autopopulate the fields, select **Upload**, browse to the CSV file on the USB drive, select the CSV file, and select **Open**.
 - » To manually populate the fields, enter the access key and secret access key.
8. Select **Save** to add the storage connection.
9. If you uploaded a CSV file, disconnect the USB drive:
 - a. In the taskbar, select **USB Drive**, and then select **Eject**.
 - b. Detach the USB drive from the instrument.

Add a GCS Storage Connection

1. If you are importing the access key and secret access key, prepare the CSV file:
 - a. Save the CSV file to a compatible USB drive.
 - b. Connect the USB drive to a USB port on the side or back of the instrument.
2. On the taskbar, select **Settings**.
3. Select the **Storage** tab.
4. Select **Add Storage**, and then select **GCS** as the storage provider.
5. In the Name field, enter a preferred name for the storage connection.

6. Complete the following fields to configure a Cloud Storage bucket as the storage location.

Field	Instruction
Bucket Name	Enter the bucket name, such as NGS-GCS-123.
GCS Prefix	Enter the prefix for the bucket, such as bucket-name.

—Only the Bucket Name field is required.—

7. Complete the Access Key and Secret Access Key fields using either method:
- » To autopopulate the fields, select **Upload**, browse to the CSV file on the USB drive, select the CSV file, and select **Open**.
 - » To manually populate the fields, enter the access key and secret access key.
8. Select **Save** to add the storage connection.
9. If you uploaded a CSV file, disconnect the USB drive:
- a. In the taskbar, select **USB Drive**, and then select **Eject**.
 - b. Detach the USB drive from the instrument.

Add an SMB Storage Connection

1. On the taskbar, select **Settings**.
2. Select the **Storage** tab.
3. Select **Add Storage**, and then select **Local File Server (SMB)** as the storage provider.
4. In the Name field, enter a preferred name for the storage connection.
5. Complete the following fields to configure an SMB network storage location for the SMB storage connection.

Field	Instruction
Host	Enter the host network IP address or fully qualified domain name (FQDN). The Kerberos authentication protocol requires an FQDN. <ul style="list-style-type: none">• Example IP address: 1.222.333.44• Example FQDN: elembio.com
Port	Enter a port number for the file transfer service or leave blank to accept the default of port 445.
Workgroup/Domain	Enter the name of the work group or domain that the user belongs to. If you are using Kerberos authentication protocol, enter the Kerberos realm name.
Share	Enter the name of the share that makes the directory accessible to SMB.
Path	Enter the path to an existing folder where you want to output data.
User	Enter the user name for the service user.
Password	Enter the password for the service user.

—All fields except Port and Path are required. Certain server configurations require a work group or domain.—

6. Select **Save** to add the storage connection.

Add a USB Storage Connection

1. Connect a USB drive to a USB port on the side or back of the instrument.
2. On the taskbar, select **Settings**.
3. Select the **Storage** tab.
4. Select **Add Storage**, and then select **USB Drive** as the storage provider.
5. In the USB Drive list, select the USB drive connected to the instrument.
6. In the Name field, enter a preferred name for the storage connection.
7. Select **Save** to add the storage connection.
—AVITI OS makes sure the USB drive is connected to the instrument and has write permission and sufficient storage.—
8. Disconnect the USB drive:
 - a. Select **More** (three dots) for the USB storage connection, and then select **Eject**.
 - b. Detach the USB drive from the instrument.

Disconnect a USB for a Storage Connection

1. Select **More** (three dots) for the USB storage connection.
2. Select **Eject**.
3. Detach the USB drive from the instrument.
—To reuse the USB after disconnecting, reconnect the device to a USB port. The device must maintain the same name for the system to identify the storage connection.—

Manage Storage Connections

Storage settings manage storage connections, including setting the default. Unless you reset the default storage connection, the default is the first cloud location added to the instrument. If a cloud location does not exist, the default storage connection is the first local network location.

You can verify any storage connection, but only local storage connections can be edited and deleted. If you must edit a cloud storage connection, instead create another storage connection.

Verify a Storage Connection

1. On the taskbar, select **Settings**.
2. Select the **Storage** tab.
3. For the applicable storage connection, select the three dots, and then select **Verify Storage**.
4. Wait ~20 seconds for a success message to appear, indicating a valid storage connection.
—AVITI OS indicates that the connection is connected, unverified, or partially verified with a blocked network.—
5. If AVITI OS cannot verify the storage connection, troubleshoot:
 - a. Make sure the storage connection is correctly set up.
 - For an AWS storage connection, check the IAM permissions. See the *Amazon S3 IAM Policy Template* at go.elembio.link/documentation.
 - For a GCS storage connection, check the role assigned to the HMAC key.
 - For an SMB storage connection, check the permissions associated with the users.
 - For a USB storage connection, make sure the USB is not ejected, and check that the USB name and type are correct. For USB requirements, see [Local Storage on page 70](#).
 - b. If the storage connection is correctly set up, contact Element Technical Support.

Set the Default Storage Connection

1. On the taskbar, select **Settings**.
2. Select the **Storage** tab.
3. For the applicable storage connection, select the three dots, and then select **Set as Default**.
4. When prompted, select **Set Default**.

Edit a Local Storage Connection

1. On the taskbar, select **Settings**.
2. Select the **Storage** tab.
3. Select **Edit** for the local storage connection you want to update.
—Editing a busy storage connection can affect where run data are output.—
4. On the Edit Storage Connection screen, edit any of the following fields.

Field	Instruction
Name	Enter a preferred name for the storage connection.
Workgroup/Domain	Enter the name of the work group or domain that the user belongs to. If you are using Kerberos authentication protocol, enter the Kerberos realm name.
User	Enter the user name for the service user.
Password	Enter the password for the service user.

—The Host, Share, Port, and Path fields are read-only. If you must edit these fields, create another storage connection.—

5. Select **Save** to apply the edits and update the storage connection.

Delete a Local Storage Connection

1. On the taskbar, select **Settings**.
2. Select the **Storage** tab.
3. Select **Delete** for the local storage connection you want to delete.
4. When prompted, select **Delete**.

—AVITI OS does not allow you to delete a busy storage connection.—

Update the Software

AVITI OS checks online systems for a new software version one time per day. A notification appears when a new version is available and prompts you to perform the update, which occurs remotely and takes ~15 minutes.

For offline systems, Element notifies you of an update and provides the files needed for a manual update. Manual updates are only available for systems in offline mode.

Perform a Remote Update

1. Make sure the AVITI System is not performing a run or wash.
2. On the taskbar, select **Settings**, and then select **Update Software**.
3. When prompted, select **Update Now** to start the update.
—When the update is complete, the system automatically restarts.—
4. After the system restarts, select **Notifications** to view a notification confirming success.
5. If the update was unsuccessful, contact Element Technical Support.
—AVITI OS reverts to the previous version so you can continue operation.—

Perform a Manual Update

1. Save the files that Element provides for the update at the root level of a USB drive.
2. Connect the USB drive to a USB port on the side or back of the instrument.
3. Make sure the AVITI System is not performing a run or wash.
4. On the taskbar, select **Settings**.
5. Under Software Update, in the USB Drive list, select the USB drive that contains the update files.
6. When prompted, select **Update Now** to perform the update.
—When the update is complete, the system automatically restarts.—
7. After the system restarts, select **Notifications** to view a notification confirming success.
8. If the update was unsuccessful, contact Element Technical Support.
—AVITI OS reverts to the previous version so you can continue using the system.—
9. Disconnect the USB drive:
 - a. On the taskbar, select **USB Drive**, and then select **Eject**.
 - b. Detach the USB drive from the instrument.

Manage an Offline System

AVITI OS allows you to export log files and password-protect the system. Both features are unique to offline mode and help manage an offline system.

For software update instructions, see [Update the Software on page 78](#).

Exporting Log Files

Offline systems support the export of log files using two methods:

- **Automatic export**—Configure AVITI OS to automatically export log files to a local storage location every hour for telemetry purposes. For help connecting exported log files to telemetry, contact Element Technical Support.
- **Manual export**—Export log files to a USB drive as needed to provide troubleshooting resources to Element Technical Support.

By default, automatic export is disabled and AVITI OS does not export any log files. When exporting log files to a USB drive, a solid-state drive (SSD) offers significant time savings compared to a flash drive.

Enable Automatic Export of Log Files

1. If necessary, add a local storage connection to export log files to. For instructions, see [Add Storage Connections on page 73](#).
2. On the taskbar, select **Settings**.
3. Select the **General** tab, and then select **Set Up Automatic Export**.
4. In the Storage Connection list, select a local storage connection.
5. Select **Save** to enable automatic export.
6. Transfer the exported log files to an internet-accessible location for telemetry.
7. Delete transferred files from the storage location.
—Each automatic export adds log files to the storage location without replacing or removing existing files.—

Disable Automatic Export of Log Files

1. On the taskbar, select **Settings**.
2. Select the **General** tab.
3. Under Export Log Files, select **Disable** to stop automatically exporting log files.

Change the Automatic Export Location

1. On the taskbar, select **Settings**.
2. Select the **General** tab.
3. Under Export Log Files, select **Edit**.
4. In the Storage list, select a local storage location to export log files to.
5. Select **Save** to reset the location.

Manually Export Log Files

1. Connect a USB drive to a USB port on the side or back of the instrument.
2. On the taskbar, select **Settings**.
3. Select the **General** tab.
4. Under Export Log Files, select **Manual**.
5. In the USB Drive list, select the USB drive connected to the instrument.
6. Select **Export Logs**.
—AVITI OS exports log files from the last 30 days to the USB drive.—
7. Disconnect the USB drive:
 - a. On the taskbar, select **USB Drive**, and then select **Eject**.
 - b. Detach the USB drive from the instrument.
8. Upload the log files to the location that Element Technical Support provides.

Manage Passwords

User settings manage passwords for offline systems and online systems with local authentication. Offline systems support setting, changing, resetting, and removing passwords. An online system supports password reset and removal only.

**NOTE**

Resetting or removing a password requires assistance from Element Technical Support.

Set a Password

1. On the taskbar, select **Settings**.
2. Select the **User** tab.
3. In the Password field, enter a new password.
—The field accepts ≥ 4 alphanumeric and special characters, excluding spaces.—
4. In the Confirm Password field, reenter the new password.
5. Select **Save**.
6. When prompted, select **Yes, Set Password**.

Change the Password

1. On the taskbar, select **Settings**.
2. Select **User**.
3. In the Current Password field, enter the current password.
4. In the Password field, enter a new password.
—The field accepts ≥ 4 alphanumeric and special characters, excluding spaces.—
5. In the Confirm Password field, reenter the new password.
6. Select **Save** to apply the new password.

Reset a Lost Password

1. On the login screen, select **Forgot Password**.
2. Select **Generate** to display a password reset token and the instrument serial number.
3. Contact Element Technical Support and provide the token and serial number.
—Element Technical Support emails you a single-use password reset file.—
4. Save the password reset file at the root level of a USB drive. Do not rename the file or save it in a folder.
5. Connect the USB drive to a USB port on the side or back of the instrument.
6. Select **Next**.
7. Select **Load Reset File** to upload the password reset file, which removes the password from the system.
8. In the Password field, enter a new password.
—The field accepts ≥ 4 alphanumeric and special characters, excluding spaces.—
9. In the Confirm Password field, reenter the new password.
10. Select **Reset Password** to apply the new password and return to the login screen.
11. Sign in to the system using the new password.
12. Disconnect the USB drive:
 - a. On the taskbar, select **USB Drive**, and then select **Eject**.
 - b. Detach the USB drive from the instrument.
13. Discard the password reset file.

Remove the Password

1. On the taskbar, select **Settings**.
2. Select **User**, and then select **Remove Password**.
3. When prompted, select **Yes, Remove Password**.
4. Select **Generate** to display a password reset token and the instrument serial number.
5. Contact Element Technical Support and provide the token and serial number.
—Element Technical Support emails you a single-use password reset file.—
6. Save the password reset file at the root level of a USB drive. Do not rename the file or save it in a folder.
7. Connect the USB drive to a USB port on the side or back of the instrument.
8. Select **Next**.
9. Select **Load Reset File** to upload the password file, which removes the password from the system.
10. Disconnect the USB drive:
 - a. On the taskbar, select **USB Drive**, and then select **Eject**.
 - b. Detach the USB drive from the instrument.
11. Discard the password reset file.

CHAPTER 9

Troubleshooting

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General Troubleshooting

Error messages communicate hardware or software problems and describe both the problem and resolution. General troubleshooting resolves other problems that can occur during system initialization, run setup, and sequencing. If a problem persists, contact Element Technical Support.

A power cycle resolves many common problems. For instructions, see [Power Cycle the System on page 61](#).

Initialization Problems

Power cycle the system to resolve the following initialization problems:

- After turning on the instrument, the monitor does not display AVITI OS.
- The initialization sequence is incomplete, so the loading screen remains after ~10 minutes.

Run Setup Problems

Problem	Resolution
The flow cell is cracked, scratched, or otherwise damaged.	Contact Element Technical Support.
The lid does not engage when a flow cell is on the nest.	Remove the flow cell and wipe the nest. Inspect the flow cell for large debris and wipe with an alcohol pad if necessary. Reload the flow cell.
AVITI OS detects a full waste bottle, but the bottle is empty.	Reload the waste bottle and make sure the waste bay is unobstructed.
AVITI OS cannot detect a loaded sequencing cartridge or waste bottle.	Follow the onscreen prompt to reload the sequencing cartridge or waste bottle. Make sure the applicable bay, reagent or waste, is unobstructed, and that the sequencing cartridge is contained within a sequencing basket.
The system cannot scan or detect a barcode on the sequencing cartridge, buffer bottle, or flow cell.	Follow the onscreen prompt to reload the consumable or continue without scanning by entering the consumable part number.
The flow cell version is incompatible with the sequencing cartridge.	Load a flow cell that is the same version as the cartridge.
The reagent bay contains condensation.	Dry the inside of the reagent bay with a clean, dry microfiber cloth. Clean to the back of the bay, avoiding sensors and cables.

Problem	Resolution
The reagent or waste bay contains liquid.	See Clean Spills and Leaks on page 88 .
Liquid is spilling from the front or bottom of the instrument.	
The nest is wet.	

Sequencing Problems

Problem	Resolution
The software, instrument, keyboard, or mouse stopped operating.	Power cycle the system.
Sequencing continues after you stop a run.	Wait for the run to stop. AVITI OS waits for a safe point to stop the run, which can take several minutes to ~2 hours depending on the run stage.
The run folder is missing data.	Make sure the user interface indicates that data are uploading and wait for the upload to complete. <ul style="list-style-type: none">• A slow connection delays data transfer.• Data transfer failure prompts a notification.
Polony density is lower or higher than expected.	Contact Element Technical Support or stop the run. See Stop an Active Run on page 85 .
The assigned or perfect match metrics are lower than expected.	Make sure that the index sequences recorded in the run manifest are correct.
The samples with low representation metric is higher than expected.	Select Sample Details to view the samples with low representation. Make sure that the index sequences recorded in the run manifest and the pooling concentration are correct.
The Q30 percentage is lower than expected.	Contact Element Technical Support.
The PhiX error rate is higher than expected.	
The flow cell contains very few colonies or no colonies.	
The user interface is frozen.	

Cancel Runs and Washes

AVITI OS displays the following buttons for canceling runs and washes:

- **Discard**—Appears on every run and wash setup screen and cancels setup. The button is enabled when you can discard the run or wash without compromising the consumables.
- **Stop**—Appears on the Home screen and cancels an active run. The button is always enabled so you can free the instrument when run parameters are incorrect, data quality is poor, or a hardware problem occurs.

Discard Run Setup

1. On any run setup screen before priming, select **Discard**.
2. When prompted to confirm the discard, select an option:
 - » **Unlock Door A** or **Unlock Door B**—Discard the run and save the sequencing cartridge.
 - » **Discard Setup**—Discard the run, delete the run, and return to the Home screen without saving the cartridge.
3. If you unlocked the door, proceed with the remaining steps.
4. Open the reagent bay door and remove the cartridge.
5. Place the cartridge on ice or refrigerate at 2°C to 8°C.
6. Place a clean, uncovered white wash tray onto the open reagent bay door.
7. Slide ~2/3 of the wash tray into the reagent bay, so the barcode edge is about flush with the entrance.
8. Add 660 ml nuclease-free water to the fill area, filling the wash tray to slightly above the upper fill line.
9. Slide the wash tray all the way into the reagent bay until it stops.
10. Close the reagent bay door.
 - AVITI OS deletes the run setup and returns to the Home screen.—
11. Set up a new run and use the cartridge within **4 hours**.

Discard Wash Setup

1. On any wash setup screen, select **Discard**.
2. When prompted to confirm the discard, select **Discard Setup**.
 - AVITI OS deletes the wash setup and returns to the Home screen.—

Stop an Active Run

Stopping an active run is a two-part process: stop the run, then perform a ~60-minute recovery wash to remove residual library from the fluidic system. To start the recovery wash, the other side must be idle.



CAUTION

Stopping a run is *final*. You cannot resume a stopped run or reuse any of the consumables.

Stop the Run

1. On the applicable side of the Home or Run Details screen, select **Stop**.

2. When prompted, select **Yes, Stop Run**.
—AVITI OS finishes the current step, terminates the run, and returns to the Home screen.—
3. If the other side is sequencing or washing, wait for the run or wash to complete.
4. Proceed to [Prepare Wash 2 Solution](#) and complete the recovery wash.

Prepare Wash 2 Solution

1. Gather the following materials:
 - » 2 L bottle
 - » Nuclease-free water
 - » Pipette controller
 - » Serological pipette
 - » Tween 20
 - » Used flow cell
 - » White wash tray—A used flow cell might already be in the instrument.—
2. Add 1.5 L nuclease-free water to a new 2 L bottle.
3. Attach a new serological pipette to a pipette controller.
4. Add 4.5 ml Tween 20 to the bottle to prepare 1.5 L 0.3% Tween 20.
5. Label the bottle **Wash 2 Solution**.
6. Cap the bottle and invert several times to mix.
7. Set aside Wash 2 Solution at room temperature.

Initiate a Recovery Wash

1. On the Home screen, select **New Run**.
2. If AVITI OS prompts that the flow cell is missing, load a *used* flow cell:
 - a. Select **Open Nest**.
 - b. Place the used flow cell onto the nest and close the lid.
 - c. Select **Close Nest**.
3. Select which side to wash:
 - » **Side A**—Set up a recovery wash on side A.
 - » **Both**—Set up recovery washes on sides A and B.
 - » **Side B**—Set up a recovery wash on side B.
4. Select **Wash**, and then select **Recovery**.
5. Select **Next** to proceed to the Load Wash 2 screen.

Load Wash 2 Solution

1. Open the reagent bay door.
2. Remove the buffer bottle and sequencing basket from the reagent bay. Set aside both materials.

3. Place a clean, uncovered white wash tray onto the open door.
4. Slide ~2/3 of the wash tray into the reagent bay, so the barcode edge is about flush with the entrance.
5. Add 660 ml freshly prepared Wash 2 Solution to the fill area, filling the wash tray to slightly above the upper fill line.
6. Slide the wash tray all the way into the reagent bay until it stops.
7. Close the reagent bay door.
8. Select **Next** to proceed to the Empty Waste screen.
9. [Optional] Store leftover Wash 2 Solution at 2°C to 8°C for ≤ 2 weeks.

Empty Waste and Run Wash 2

1. Open the waste bay door.
2. Unscrew the transport cap from the cap holder above the waste bay.
3. Remove the waste bottle from the waste bay and close the transport cap.
4. [Optional] Insert a funnel into a waste receptacle. Make sure the funnel is secure.
5. Open the transport cap and the vent cap.
6. Support the waste bottle with both hands and empty the waste:
 - a. Position the bottle over the funnel or waste receptacle.
 - If you inserted a funnel, align the handle to the inner edge of the funnel.
 - If you did not insert a funnel, center the handle over the waste receptacle.
 - b. Tip the bottle forward and drain. Invert the bottle and shake to expel all droplets.
 - c. If necessary, wipe liquid off the bottle.
7. Close the vent cap and return the empty waste bottle to the waste bay.
8. Screw the transport cap onto the cap holder and close the waste bay door.
9. Select **Next** to open the Run Wash 2 screen and automatically start the wash.
10. When the wash is complete, select **Next** to proceed to the Remove Tray screen.

Unload the Wash Tray

1. When prompted, open the reagent bay door and remove the wash tray.
—Residual wash solution in the wash tray is normal.—
2. Close the reagent bay door.
3. Select **Done** to proceed to the Home screen.
4. Leave the flow cell in the nest.
5. Discard the sequencing cartridge and buffer bottle and wash the basket. See [Discard the Cartridge and Bottle on page 55](#).

Clean Spills and Leaks

Clean the nest, waste bay, or reagent bay to recover from a leak or spill observed when setting up a run or wash. A leak or spill that occurs in the waste bay during a run causes an error and requires cleaning and contacting Element Technical Support.

If the bottom of the instrument is leaking or liquid is spilling from the front of the instrument: shut down and unplug the instrument if doing so is safe and contact Element Technical Support.

Clean the Nest

1. Dampen a microfiber cloth with isopropyl alcohol.
2. Wipe the nest with the damp microfiber cloth and allow to dry.
3. If necessary, use a polyurethane foam-tip swab to clean additional areas around the nest.
4. Resume run or wash setup.

Clean the Reagent Bay

1. Keep the reagent bay door open.
2. Remove any materials from the reagent bay and set aside.
3. Wipe the interior of the reagent bay with a damp microfiber cloth, cleaning to the back of the bay while avoiding sensors and cables.
4. Inspect the exterior of the instrument for any visible fluid. If necessary, wipe with a damp microfiber cloth.
5. Resume run or wash setup.

Clean the Waste Bay

1. Keep the waste bay door open. If the leak occurs during a run, open the door:
 - a. Wait for any runs or washes on the unaffected side to finish.
 - b. On the taskbar, select **Notifications**.
 - c. On the applicable error, select **Unlock Waste Module Door**.
 - d. Open the waste bay door.
2. Unscrew the transport cap from the cap holder on the affected side.
3. Remove the waste bottle from the waste bay and close the transport cap.



CAUTION

Waste droplets might be on the exterior of the waste bottle.

4. Inspect the waste bottle for cracks, holes, and other defects.
5. [Optional] Insert a funnel into a waste receptacle. Make sure the funnel is secure.
6. Open the transport cap and the vent cap.
7. Support the waste bottle with both hands and empty the waste:
 - a. Position the bottle over the funnel or waste receptacle.
 - If you inserted a funnel, align the handle to the inner edge of the funnel.
 - If you did not insert a funnel, center the handle over the waste receptacle.

- b. Tip the bottle forward and drain. Invert the bottle and shake to expel all droplets.
 - c. If necessary, wipe liquid off the bottle.
8. Close the vent cap, leave the transport cap open, and set aside the waste bottle.
 9. Wipe the interior of the waste bay with a damp microfiber cloth.
 10. Inspect the exterior of the instrument for any visible fluid. If necessary, wipe with a damp microfiber cloth.
 11. Return the waste bottle to the waste bay.
 - » If the bottle is defective and you have a spare, load the spare.
 - » If the bottle is defective and you do not have a spare, load the defective bottle. Do not use the affected side until the defective waste bottle is replaced.

—A run or wash on either side requires the presence of both bottles.—
 12. Screw the transport cap onto the cap holder and close the waste bay door.
 13. Resume run or wash setup. If necessary, set up a new run with new consumables and clean accessories.

CHAPTER 10

Safety and Compliance

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General Safety

Review this chapter before operating or maintaining the Element AVITI System to ensure safe, correct usage. The procedures described in this guide are tested and optimized, so any deviation can compromise results, cause personal injury, or damage the instrument. All personnel operating the instrument must be trained in correct operation and safety.



The *Element AVITI System Site Prep Guide (MA-00007)* provides delivery information and installation requirements, including instrument specifications, power specifications, and environmental conditions. A field service engineer (FSE) installs the AVITI System.

WARNING
 Do not attempt to move the instrument, which can result in injury. Only trained Element personnel are qualified to safely move the instrument.

Safety Labels

The following table lists the safety labels affixed to the instrument. The labels identify potential hazards associated with installation, service, and operation. Follow the procedures in this guide as described to avoid interactions that expose you to these hazards.

WARNING
 This product can expose you to chemicals including formaldehyde, which is known to the State of California to cause cancer, and methanol, which is known to the State of California to cause birth defects or other reproductive harm. For more information go to www.P65Warnings.ca.gov.

Potential Hazard	Label	Description
Class 4 Laser		The instrument is a Class 1 laser product that contains a Class 4 laser. See Laser Safety on page 92 .
Heat hazard		The nest has a hot surface and exposure can cause burns.

Certification Body Mark

In addition to the safety labels, the following Nemko label is affixed to the instrument. The label indicates that the instrument complies with Certification Body requirements in the US and Canada.

Figure 23: Label indicating Certification Body compliance



Laser Safety

The AVITI System is certified as a Class 1 laser product per the US Federal Product Performance Standard for Laser Products requirements described in 21 CFR Subchapter J. The exception to these requirements is the deviations described in FDA Laser Notice #56. The product is classified per IEC/EN 60825-1:2014.

 **WARNING**
Adjusting or performing procedures other than those described in this guide or other Element guides can result in hazardous radiation exposure.

Class 4 Laser

The instrument is a Class 1 laser product that contains a Class 4 laser. The Class 4 laser produces Class 4 levels of visible laser radiation, which can be hazardous to eyes and skin. Protective shells and safety interlocks prevent exposure or access to laser radiation levels that exceed Class 1 during operation, maintenance, or normal service.

The following figure depicts the label that identifies noninterlocked portions of the shells that prevent access to laser radiation. Additionally, the nest bay and both reagent bays contain barcode scanners that emit Class 1 levels of laser radiation.

Figure 24: Label identifying noninterlocked locations



Operating Conditions

Do not operate an AVITI System with bypassed interlocks, damaged shells, or any portion of the shells removed. These conditions make Class 4 levels of laser radiation possible and risk exposure to direct or reflected laser light.

Only Element service personnel, Element-authorized agents, or Element-trained personnel can perform services that require internal interlock bypass or removal of portions of the shells. If you are present during service, take the proper safety precautions to mitigate the risk of direct and reflected laser light.

Product Compliance

The AVITI System is verified as meeting the following standards:

- FCC 47 CFR Part 15, equipment authorization – radio frequency (RF) device
- IEC 61326-1, EMC/EMI requirement for lab equipment

The instrument is certified to the following additional standards:

- IEC 60825-1, Class 1 laser product classification
- IEC 61010-1, general safety requirements
- IEC 61010-2-010, requirements for lab equipment and heating materials
- IEC 61010-2-081, requirements for automatic and semi-automatic equipment
- UL 61010-1, safety requirements for electrical equipment

FCC Compliance

This device complies with part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

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Technical Support

Visit the [User Documentation page](#) on the Element Biosciences website for additional guides and the most recent version of this guide. For technical assistance, contact Element Technical Support.

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Document History

Document #	Date	Description of Change
Document # MA-00008 Rev. D	April 2023	<ul style="list-style-type: none"> • Updated software descriptions to AVITI OS v2.0.0. • Updated instructions on custom primers, run setup, reagent disposal, discarding runs, storage connections, and exporting log files. • Updated run statistic population times and added index assignment. • Updated lightbar colors to include washes, warnings, and errors. • Updated descriptions of the Home screen, run stages, settings, telemetry, run manifest, storage connections, and storage locations. • Updated the links for accessing user guides, templates, and safety data sheets. • Added a chemical exposure warning for Proposition 65. • Added an expected wait time for flexible start. • Added custom primer requirements. • Added instructions and a notification for software updates. • Added network and storage status indicators. • Added a USB storage connection and taskbar icon. • Added the High Elevation setting and removed the Dark Mode setting. • Added Element oligonucleotide contents. • Added troubleshooting for barcode scanning, flow cell compatibility, and index assignment. • Added LoopSeq for AVITI as a compatible library and kit compatibility. • Added the following Element products: AVITI 2x75 Sequencing Kit Cloudbreak (catalog # 860-00004), AVITI 2x150 Sequencing Kit Cloudbreak (catalog # 860-00003), Adept Custom Primer Set (catalog # 820-00009), and Cloudbreak PhiX Control Library, Elevate (catalog # 830-00017). • Identified the reagents in each well of a Cloudbreak cartridge. • Consolidated instructions on replacing primers, denaturing and diluting libraries, and cleaning the waste bay. • Replaced run.prodstats with AvitiRunStats.json. • Recommended a weekly power cycle. • Moved power cycle instructions from troubleshooting to maintenance. • Renamed AOS to AVITI OS and run statistics to run metrics. • Renamed AOS to AVITI OS, run statistics to run metrics, and the Workgroup field to Workgroup/Domain.

Document #	Date	Description of Change
Document # MA-00008 Rev. C	October 2022	<ul style="list-style-type: none"> • Updated software descriptions to AOS v1.2.0. • Updated run statistic population times. • Updated the read counts for approximate run output. • Updated the internet connection for local online networks to DHCP or static. • Updated navigation for the Home, Notifications, and Settings workspaces. • Updated loading concentrations for Adept libraries. • Updated spike-in recommendations for PhiX Control Library. • Updated the buffer bottle design. • Updated instrument certifications and laser labeling. • Updated trademark and patent information in the legal notice. • Renamed the Error Rate tab to PhiX Error. • Renamed the Element Adept Library Compatibility Kit to Element Adept Library Compatibility Kit v1.1. • Renamed the AVITI Sequencing Kit to AVITI 2x150 Sequencing Kit. • Added Element catalog # 860-00002 for the AVITI 2x75 Sequencing Kit. • Added Element catalog # 820-00008 for the Adept Custom Oligonucleotide Buffer Set. • Added custom primer information and instructions. • Added thaw times for the 2 x 75 sequencing cartridge. • Added error handling for exceeding the maximum number of cycles. • Divided the instructions to mix reagents and add library into two procedures. • Specified that primary analysis must be complete before setting up a run. • Replaced Windex glass cleaner with Simple Green All-Purpose Cleaner. • Marked the FAT32 USB drive as optional. • Corrected the list of library dilution consumables.

Document #	Date	Description of Change
Document # MA-00008 Rev. B	July 2022	<ul style="list-style-type: none"> • Updated software descriptions to AOS v1.1.0. • Updated reagent thawing, power cycling, and logoff instructions. • Updated run folder content: added statistics and upload files, removed log files, and updated bases file extensions. • Added requirement to protect the cartridge from light throughout storage, preparation, and run setup. • Added a GCS storage connection and an offline mode. • Added lightbar colors that indicate system status. • Added an onscreen keyboard and removed the requirement to connect a keyboard and mouse. • Removed the option to view notifications when signed out. • Renamed the settings chapter to <i>System Configuration</i> and reorganized. • Clarified the work group definition and requirement for an SMB storage connection. • Decreased the priming duration and increased maintenance, standby, and recovery wash durations. • Consolidated information on run and wash setup screens.
Document # MA-00008 Rev. A	June 2022	<ul style="list-style-type: none"> • Initial release



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EXHIBIT 25



US011459608B2

(12) **United States Patent**
Chen et al.

(10) **Patent No.: US 11,459,608 B2**
(45) **Date of Patent: Oct. 4, 2022**

(54) **HIGH PERFORMANCE FLUORESCENCE
IMAGING MODULE FOR GENOMIC
TESTING ASSAY**

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(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 0 days.

(21) Appl. No.: **17/372,362**

(22) Filed: **Jul. 9, 2021**

(65) **Prior Publication Data**
US 2021/0333211 A1 Oct. 28, 2021

Related U.S. Application Data

(63) Continuation of application No.
PCT/US2021/013696, filed on Jan. 15, 2021.
(Continued)

(51) **Int. Cl.**
C12Q 1/6869 (2018.01)
G01N 21/64 (2006.01)
(Continued)

(52) **U.S. Cl.**
CPC **C12Q 1/6869** (2013.01); **C12N 15/1006**
(2013.01); **C12Q 1/6806** (2013.01);
(Continued)

(58) **Field of Classification Search**
CPC G01N 21/6486; G01N 15/1436; G01N
15/147; G01N 15/1484; G01N 21/6456;
C12Q 1/6869
See application file for complete search history.

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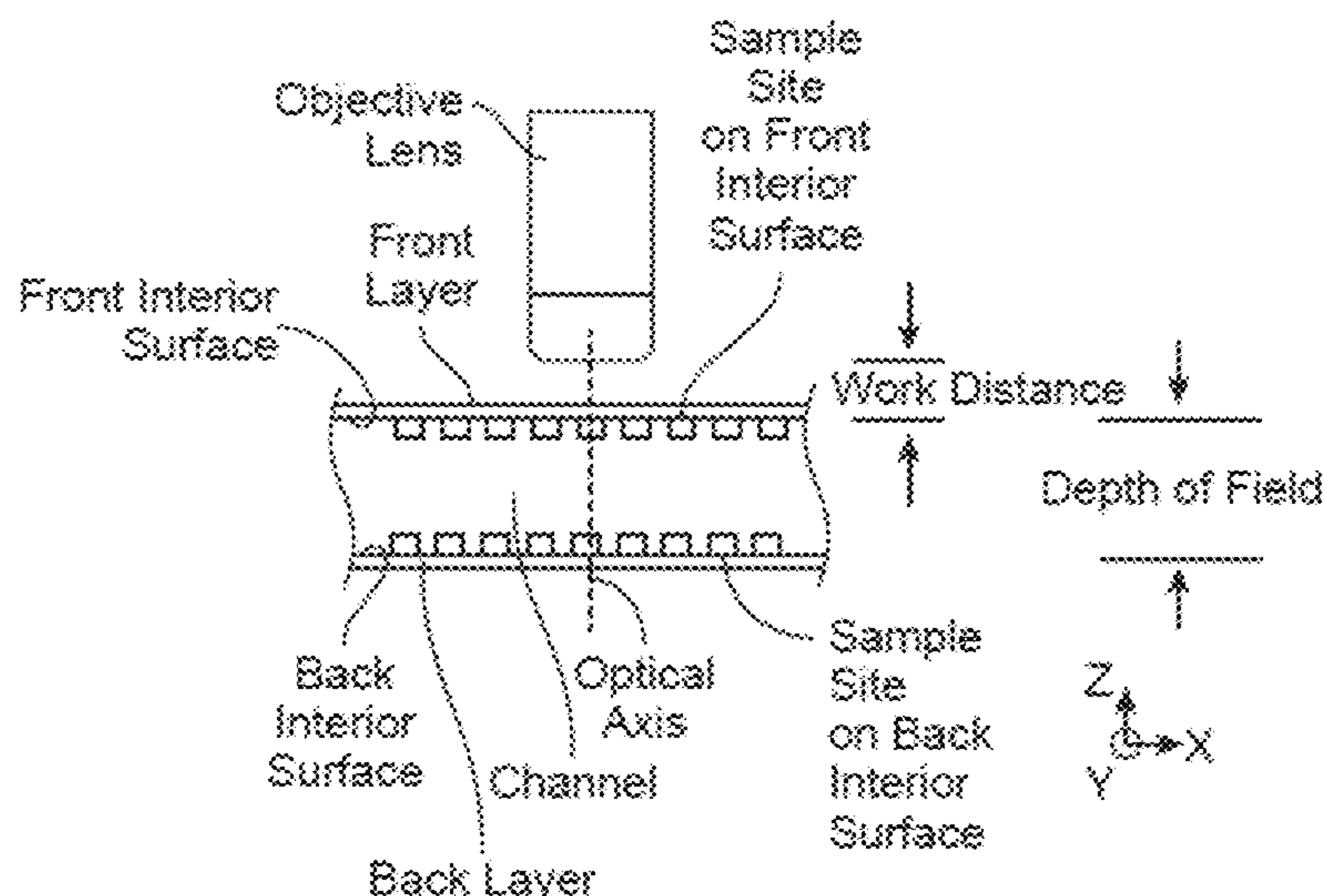
Primary Examiner — Hugh Maupin

(74) *Attorney, Agent, or Firm* — Wilson Sonsini Goodrich
& Rosati

(57) **ABSTRACT**

Fluorescence imaging system designs are described that
provide larger fields-of-view, increased spatial resolution,
improved modulation transfer and image quality, higher
spatial sampling frequency, faster transitions between image
capture when repositioning the sample plane to capture a
series of images (e.g., of different fields-of-view), and
improved imaging system duty cycle, and thus enable higher
throughput image acquisition and analysis for genomics and
other imaging applications.

20 Claims, 48 Drawing Sheets
(9 of 48 Drawing Sheet(s) Filed in Color)



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Page 2

Related U.S. Application Data

- (60) Provisional application No. 63/076,361, filed on Sep. 9, 2020, provisional application No. 62/962,723, filed on Jan. 17, 2020.
- (51) **Int. Cl.**
G01N 15/14 (2006.01)
C12N 15/10 (2006.01)
C12Q 1/6806 (2018.01)
- (52) **U.S. Cl.**
CPC *G01N 15/147* (2013.01); *G01N 15/1436* (2013.01); *G01N 15/1484* (2013.01); *G01N 21/6402* (2013.01); *G01N 21/6428* (2013.01); *G01N 21/6456* (2013.01); *G01N 21/6458* (2013.01); *G01N 21/6486* (2013.01); *G01N 2015/144* (2013.01); *G01N 2021/6439* (2013.01); *G01N 2021/6441* (2013.01); *G01N 2021/6463* (2013.01); *G01N 2201/0612* (2013.01)

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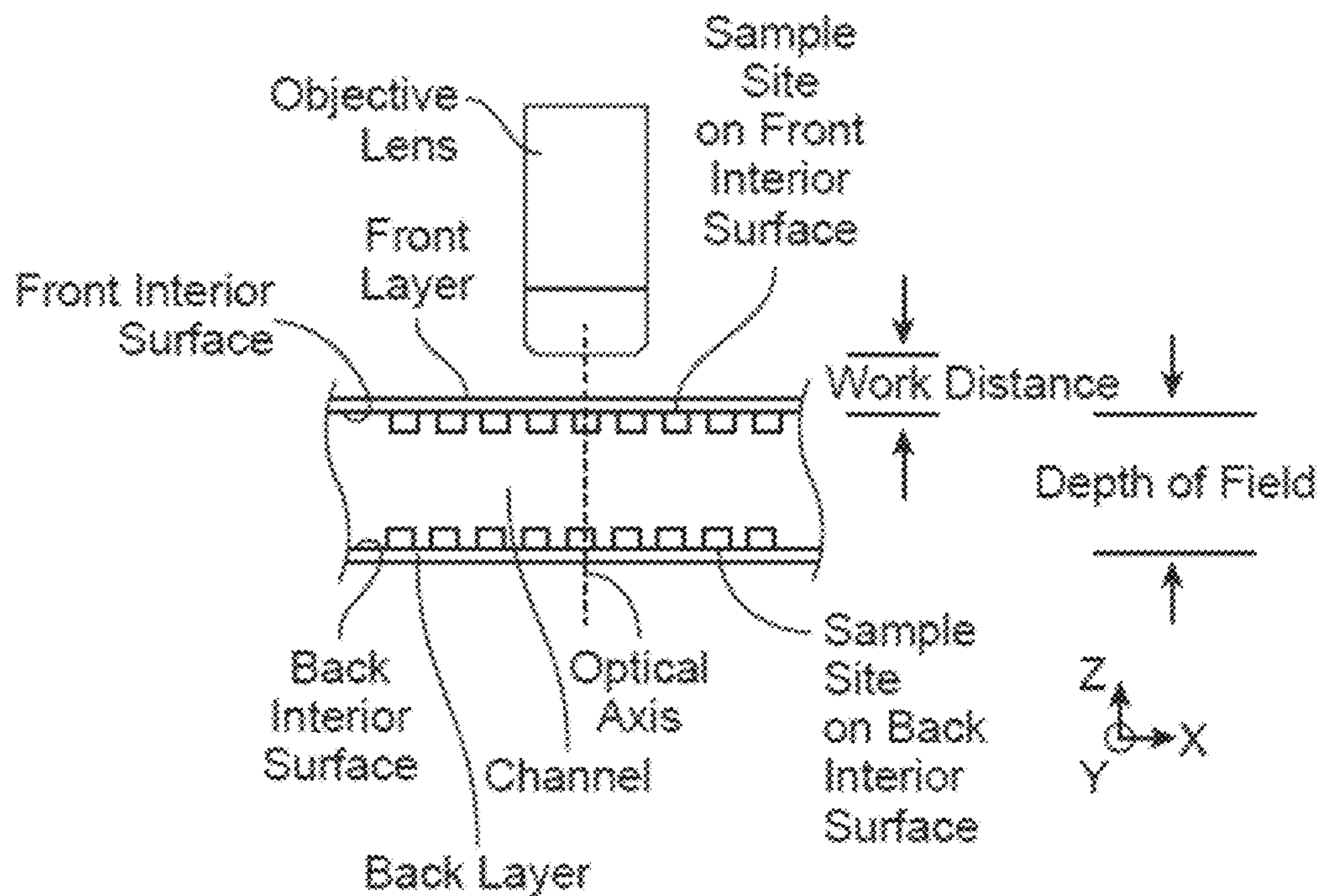


FIG. 1A

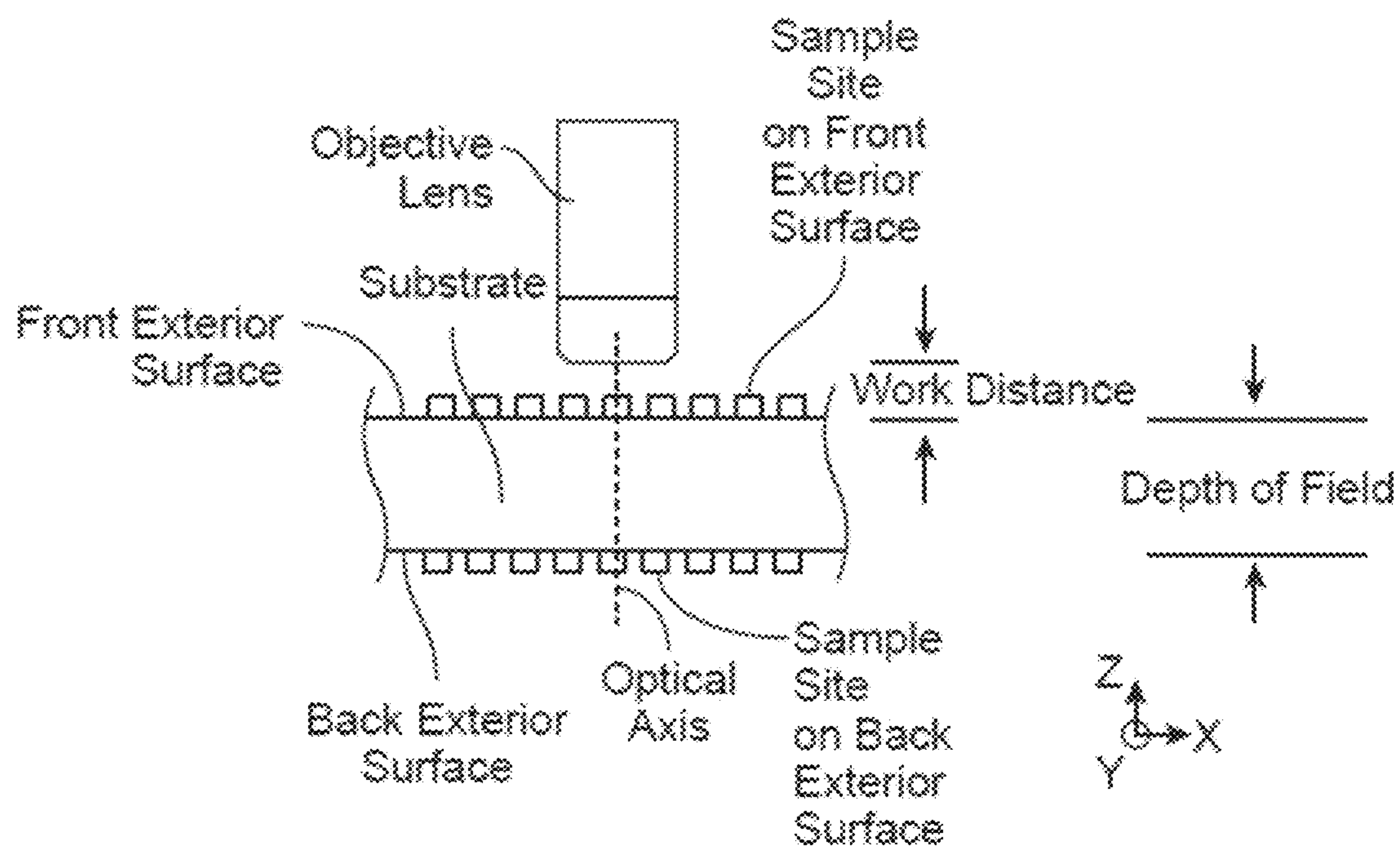


FIG. 1B

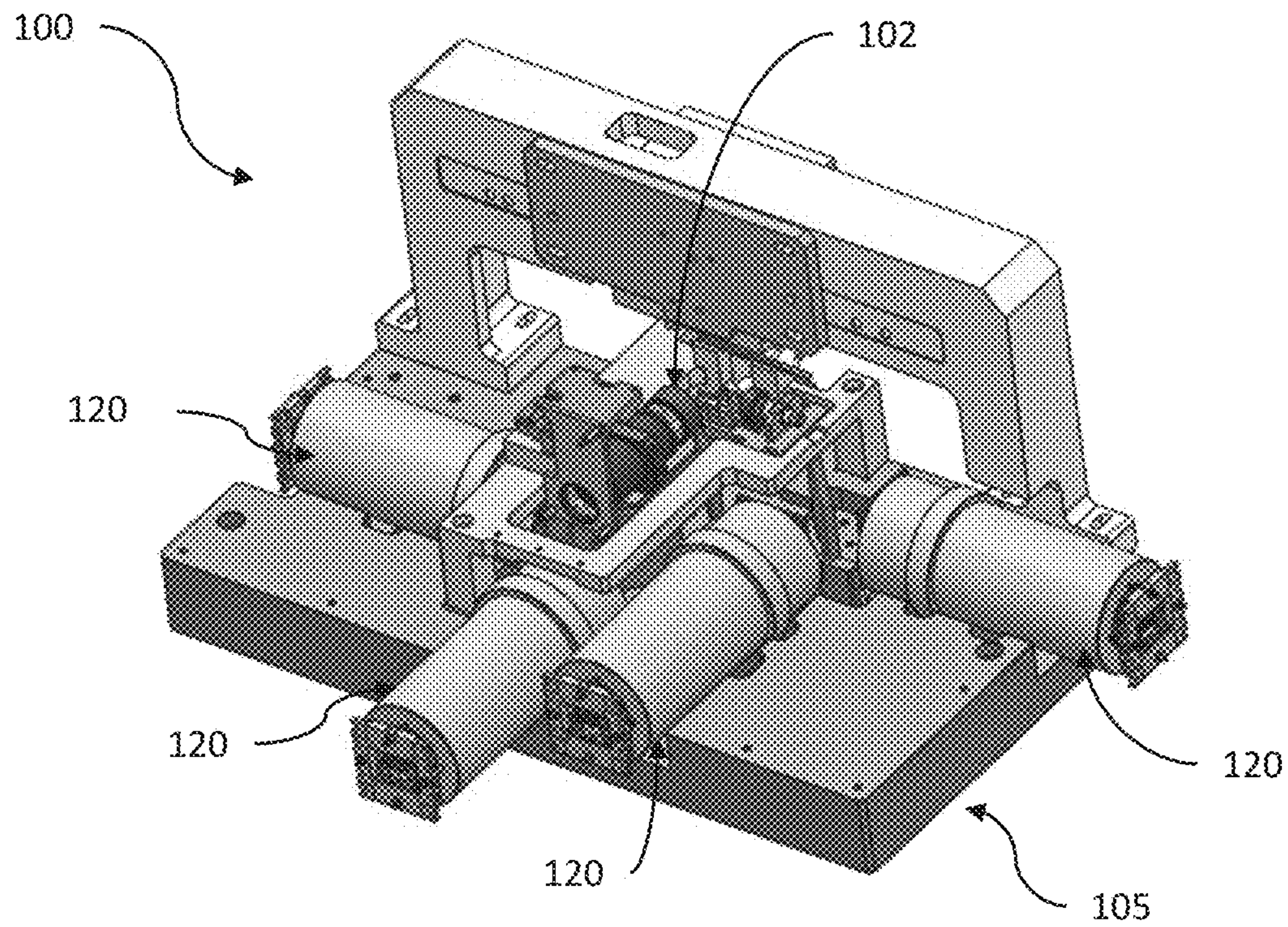


FIG. 2A

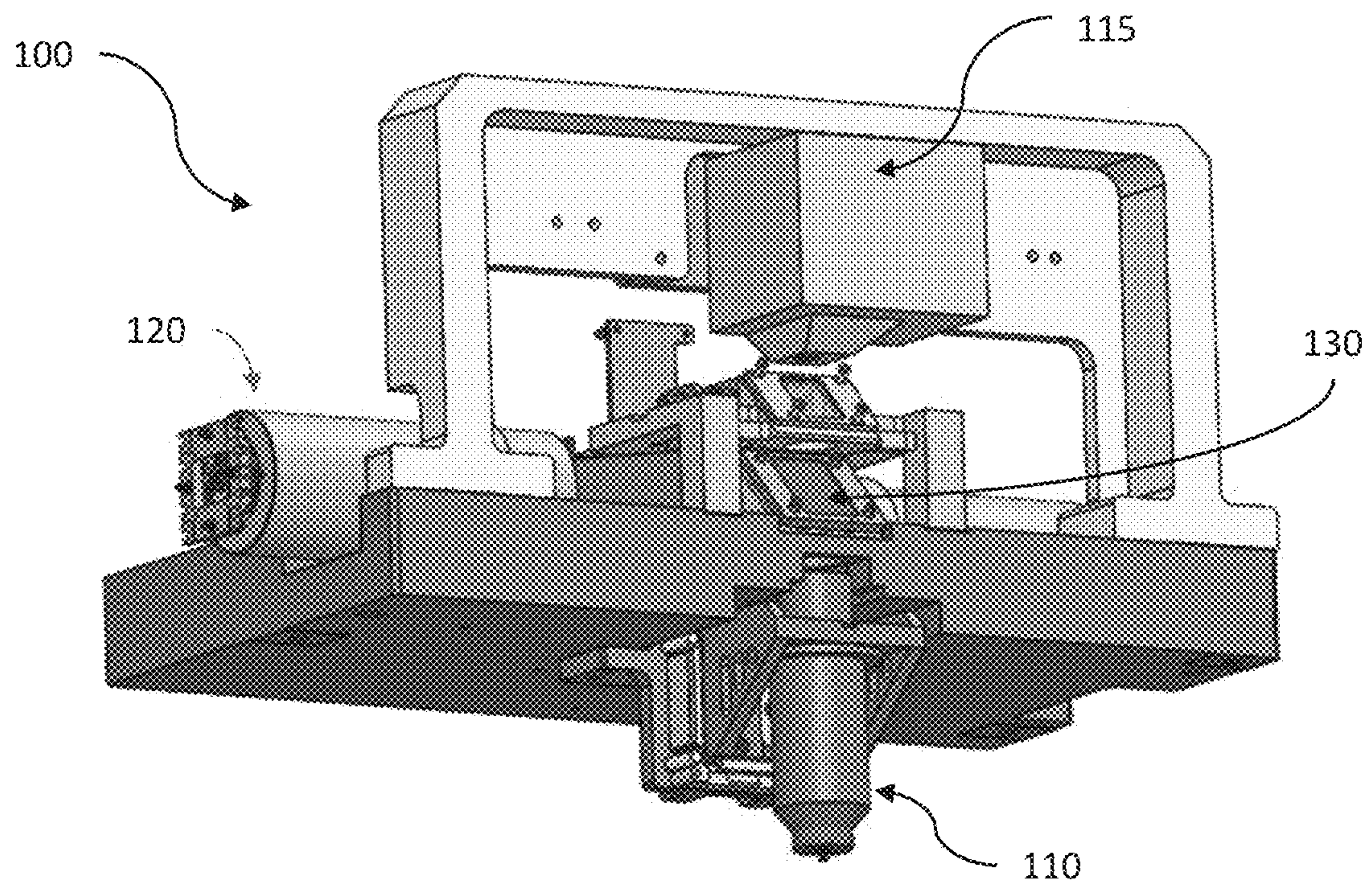


FIG. 2B

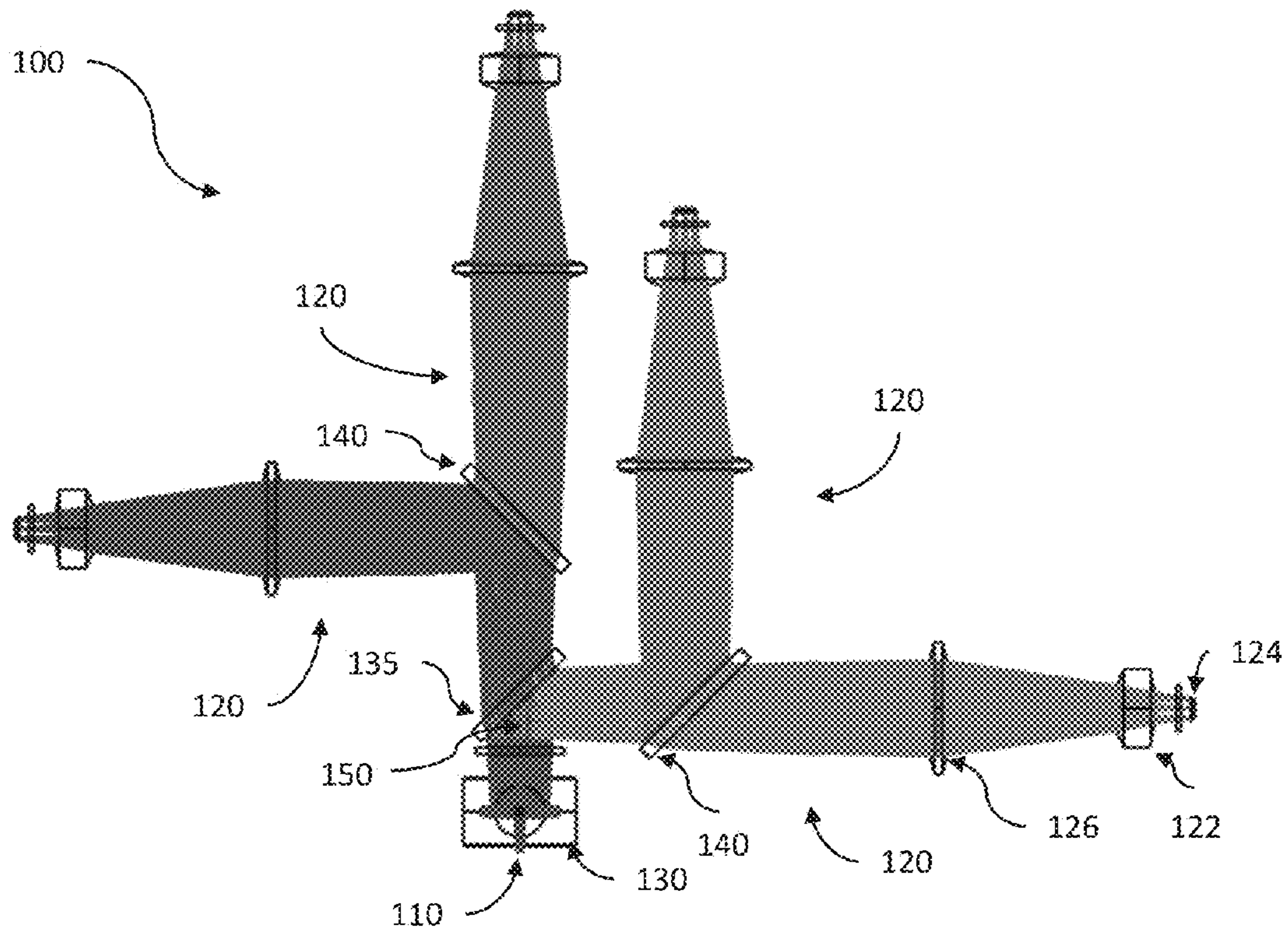


FIG. 3A

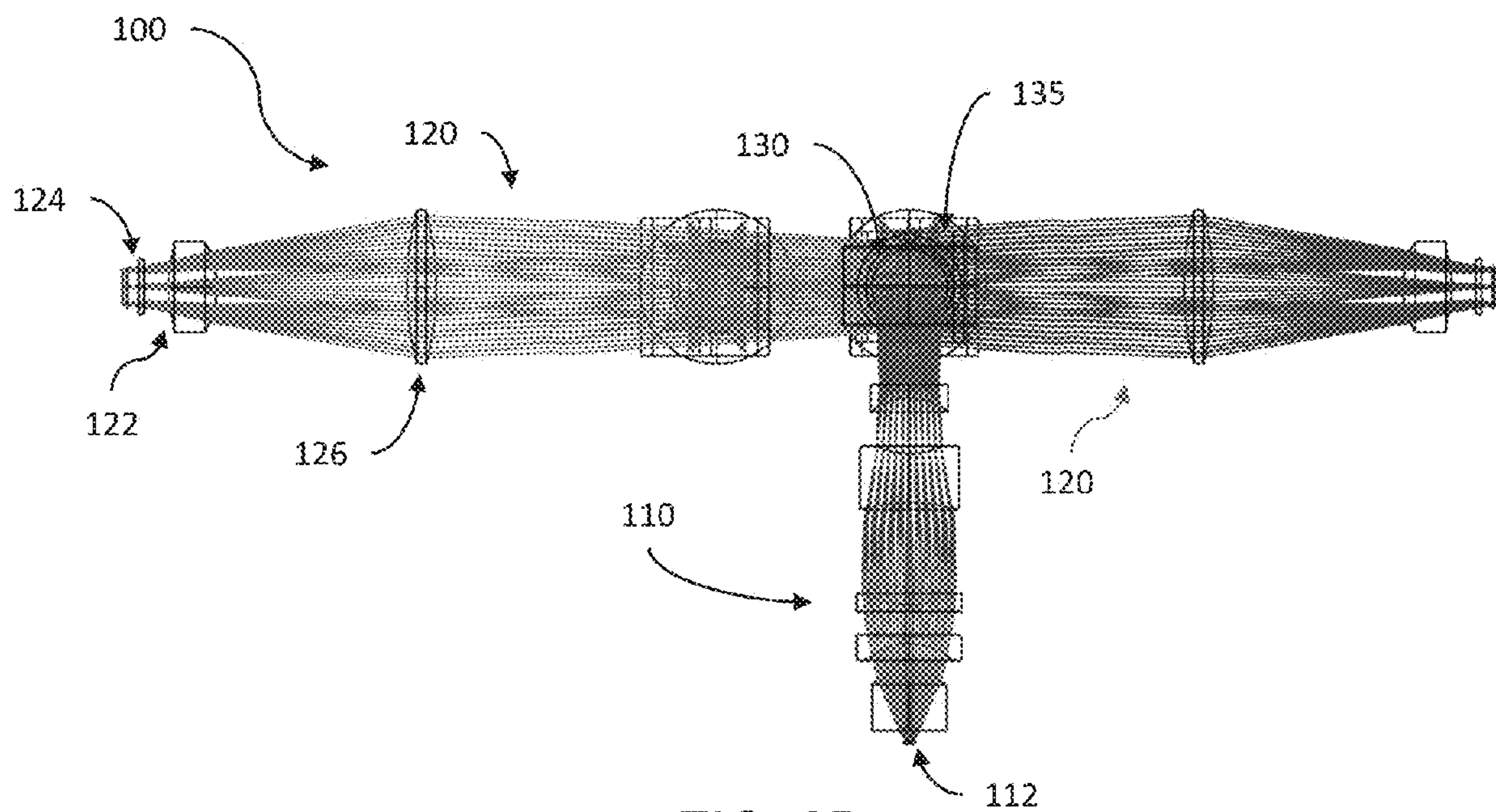


FIG. 3B

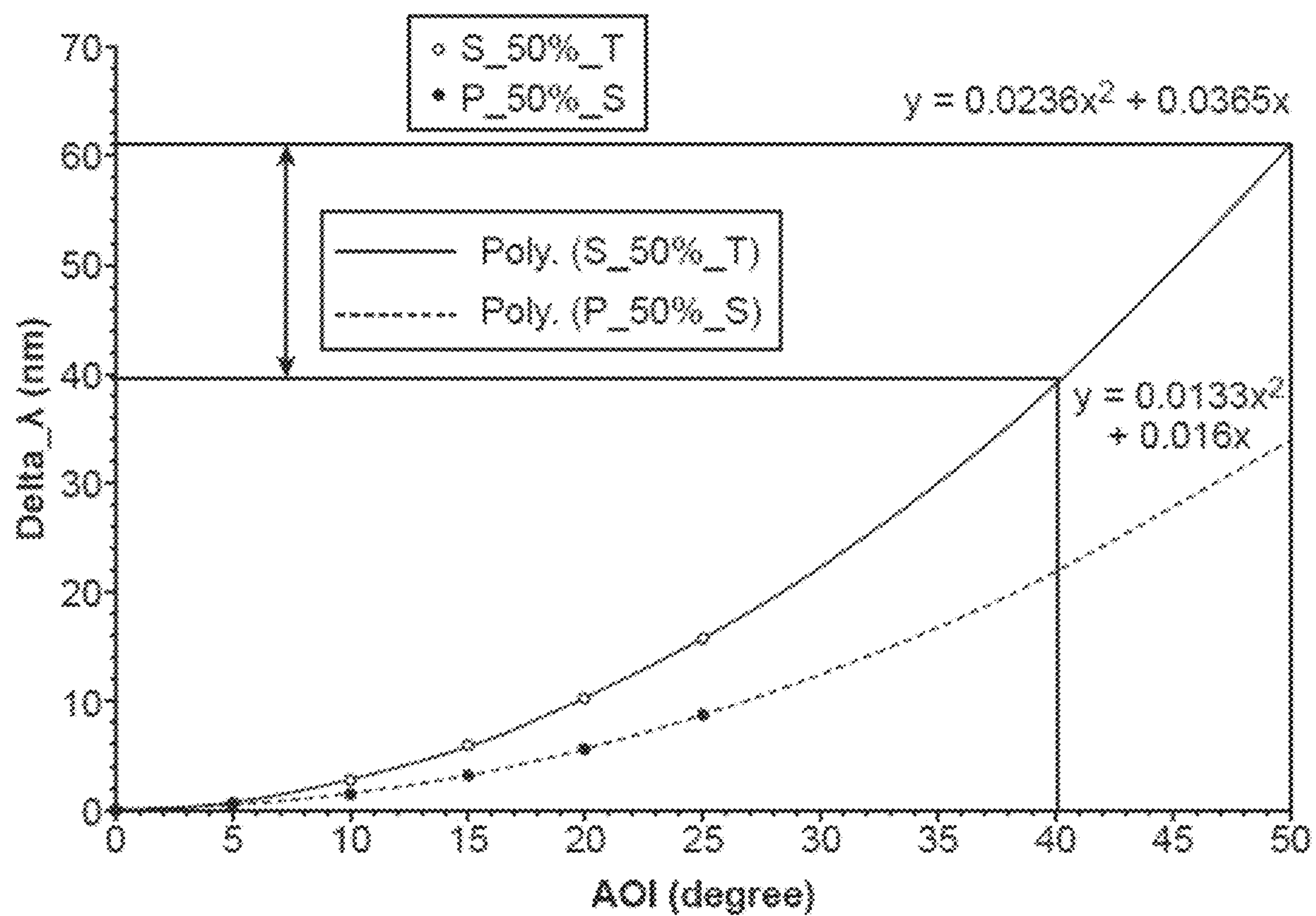


FIG. 4

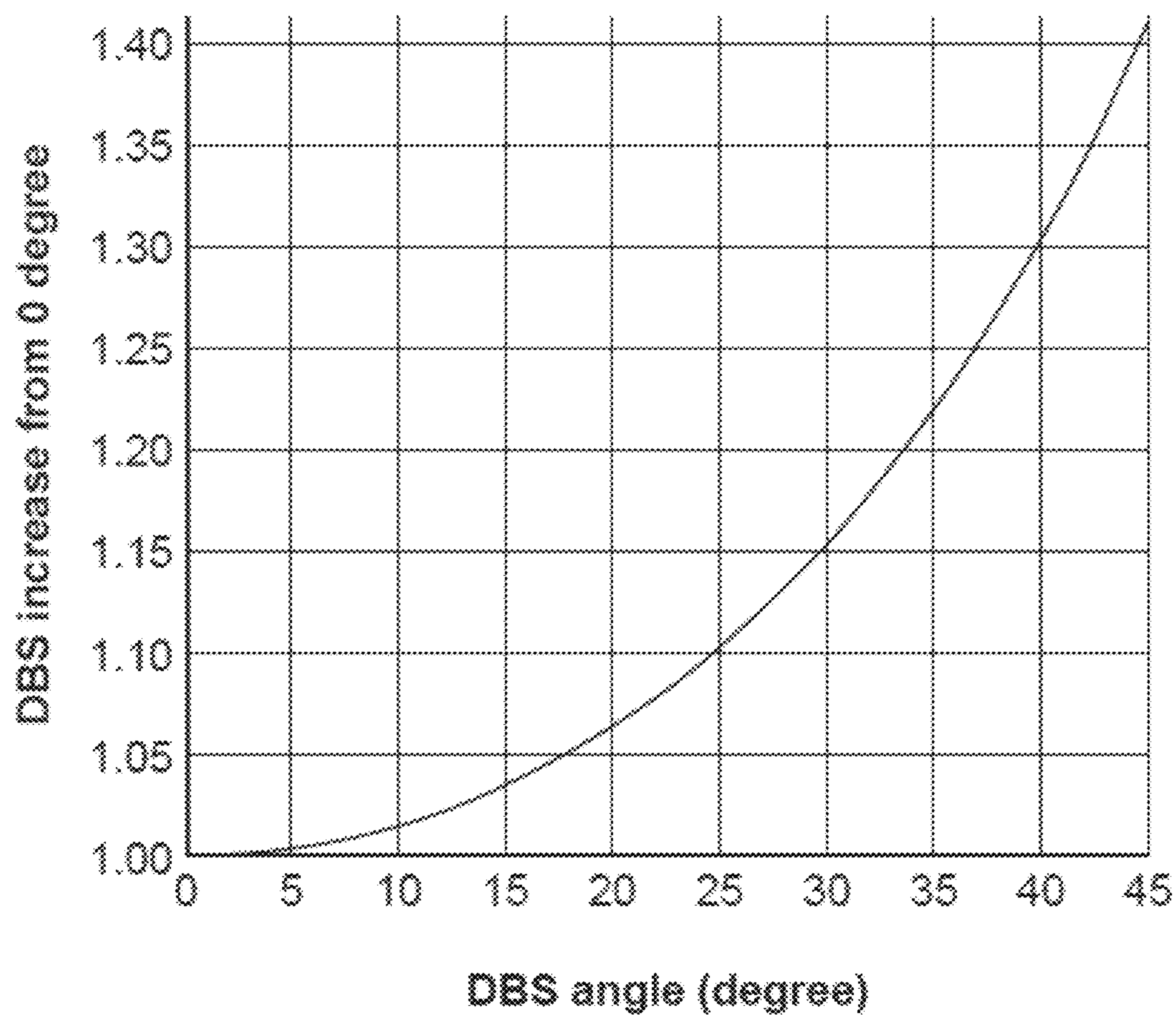
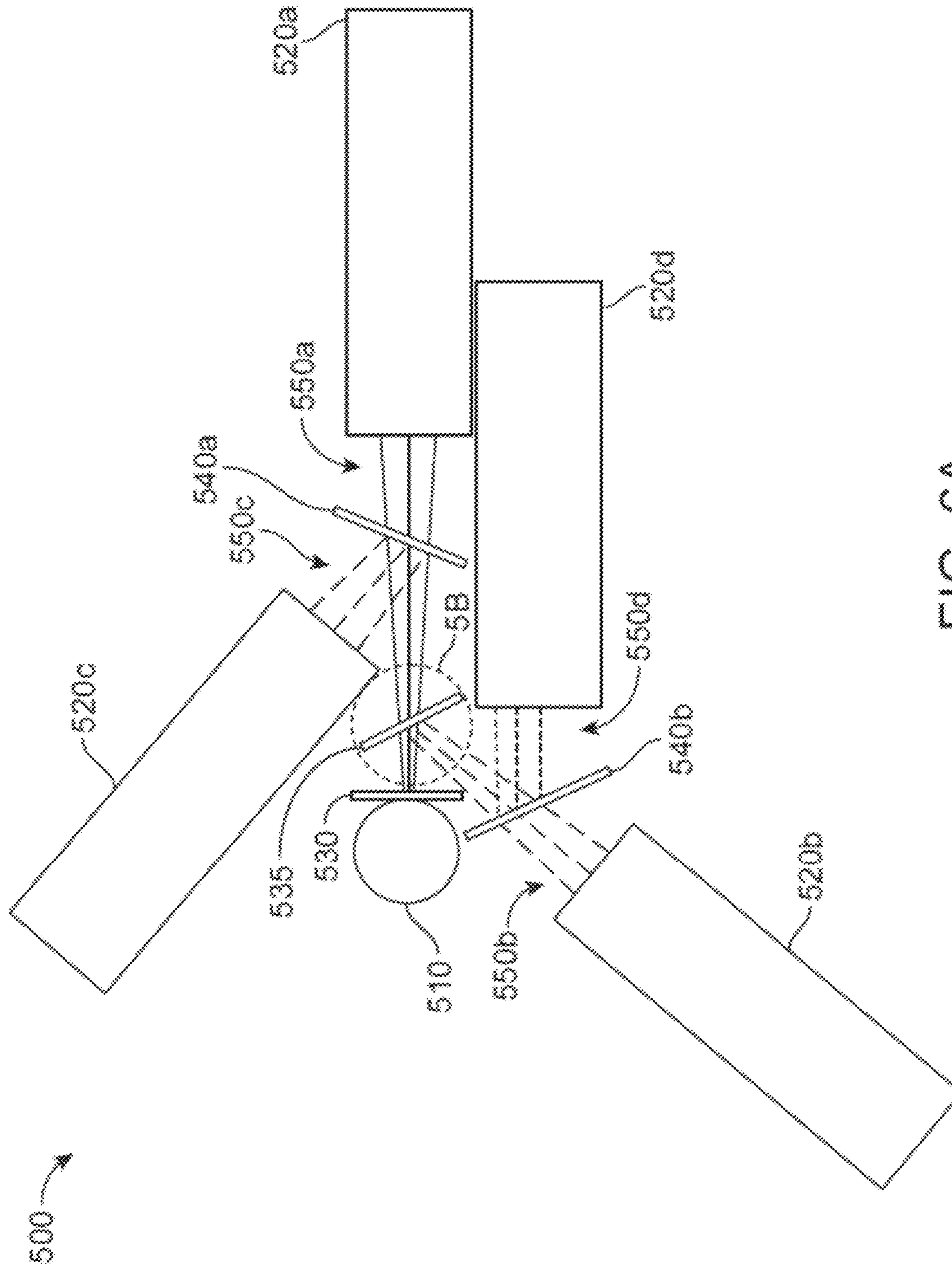


FIG. 5



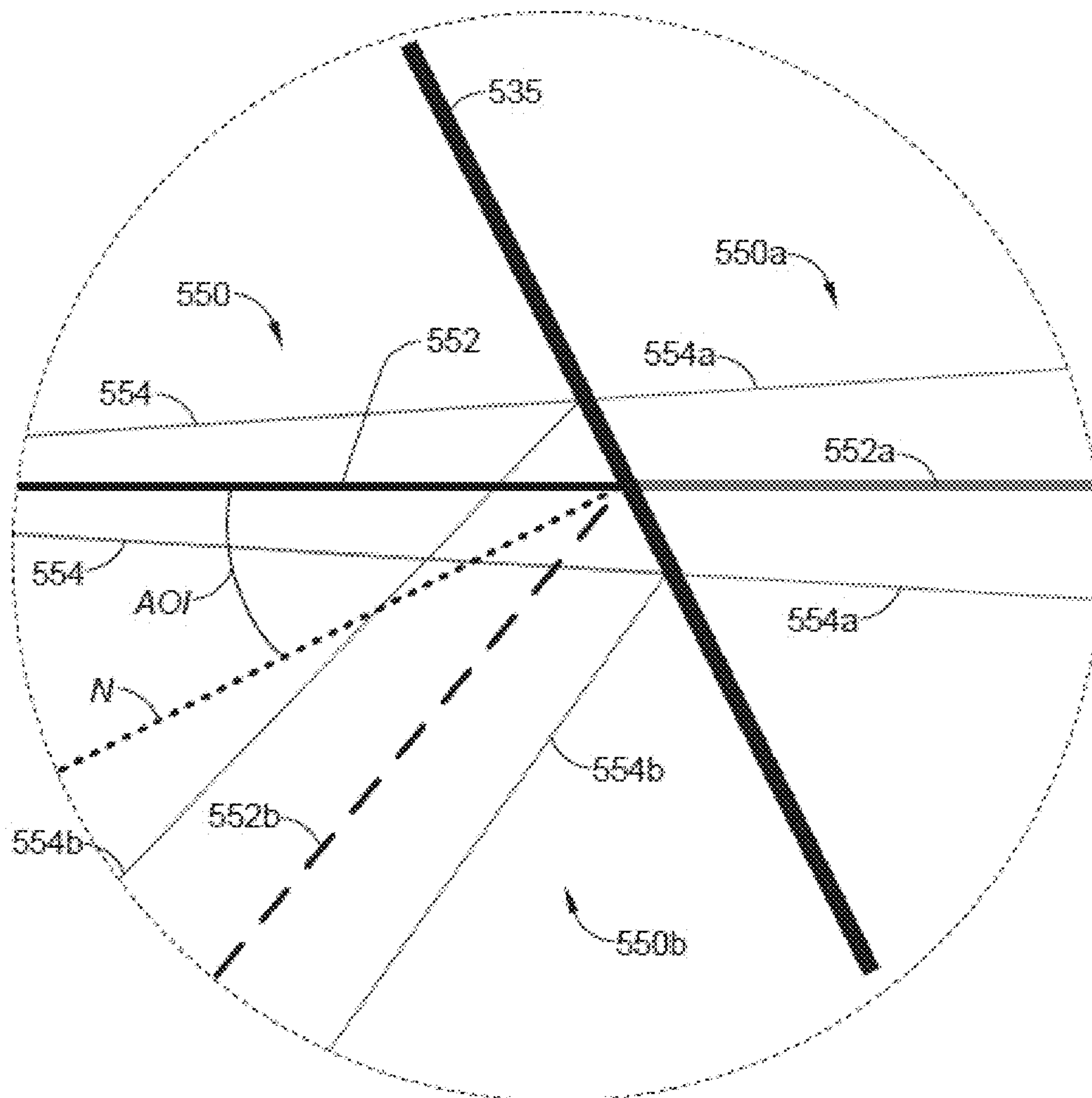


FIG. 6B

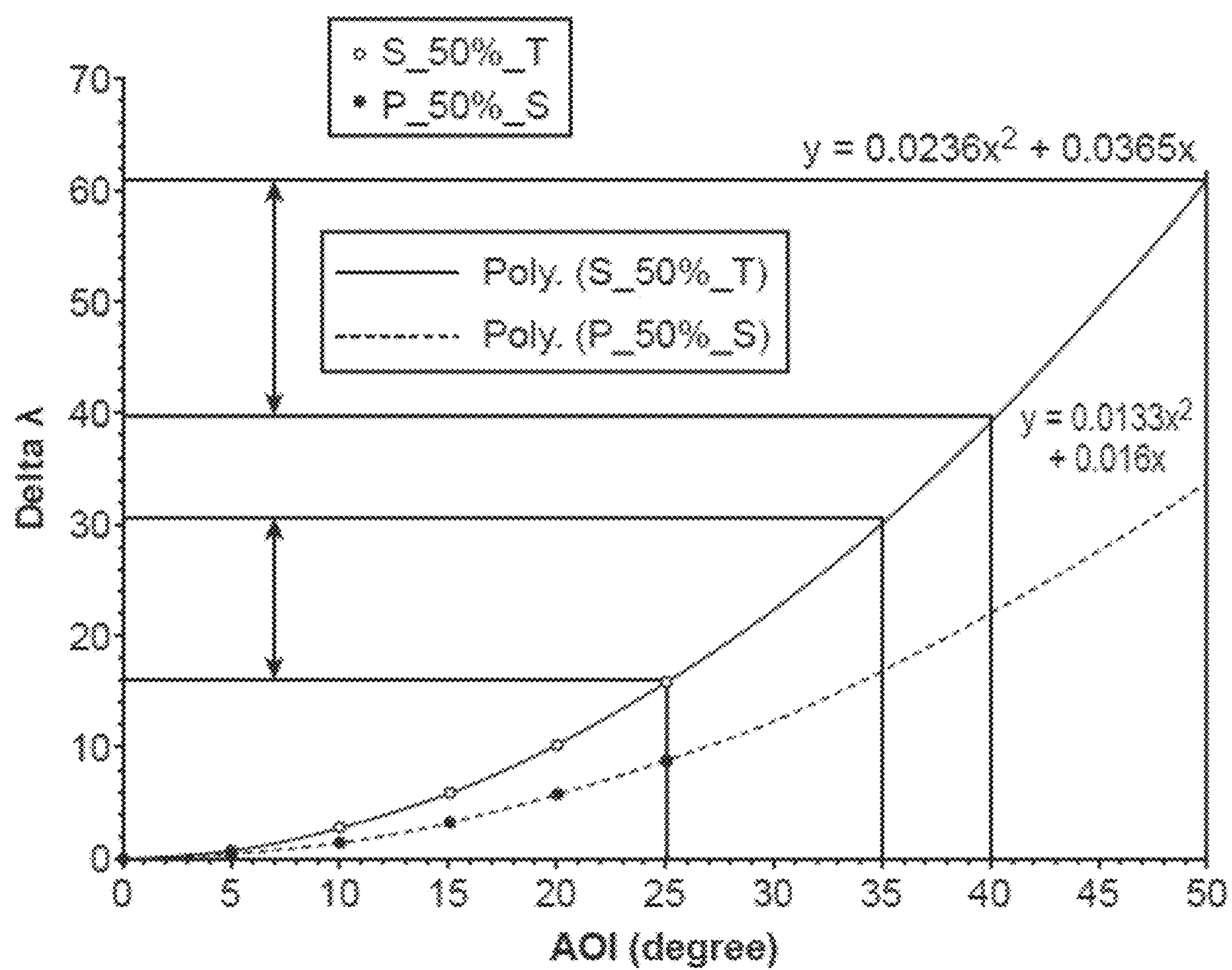


FIG. 7

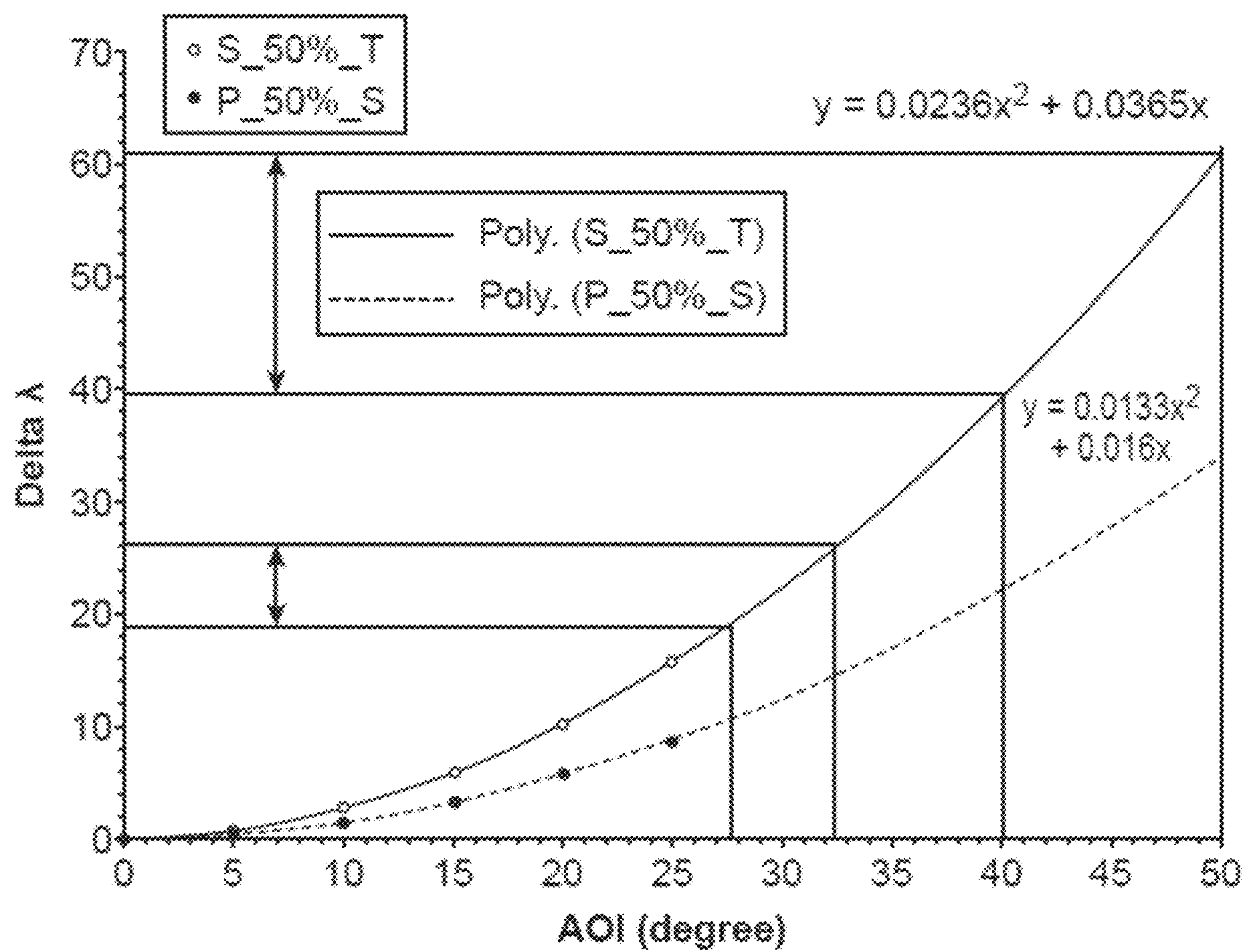


FIG. 8

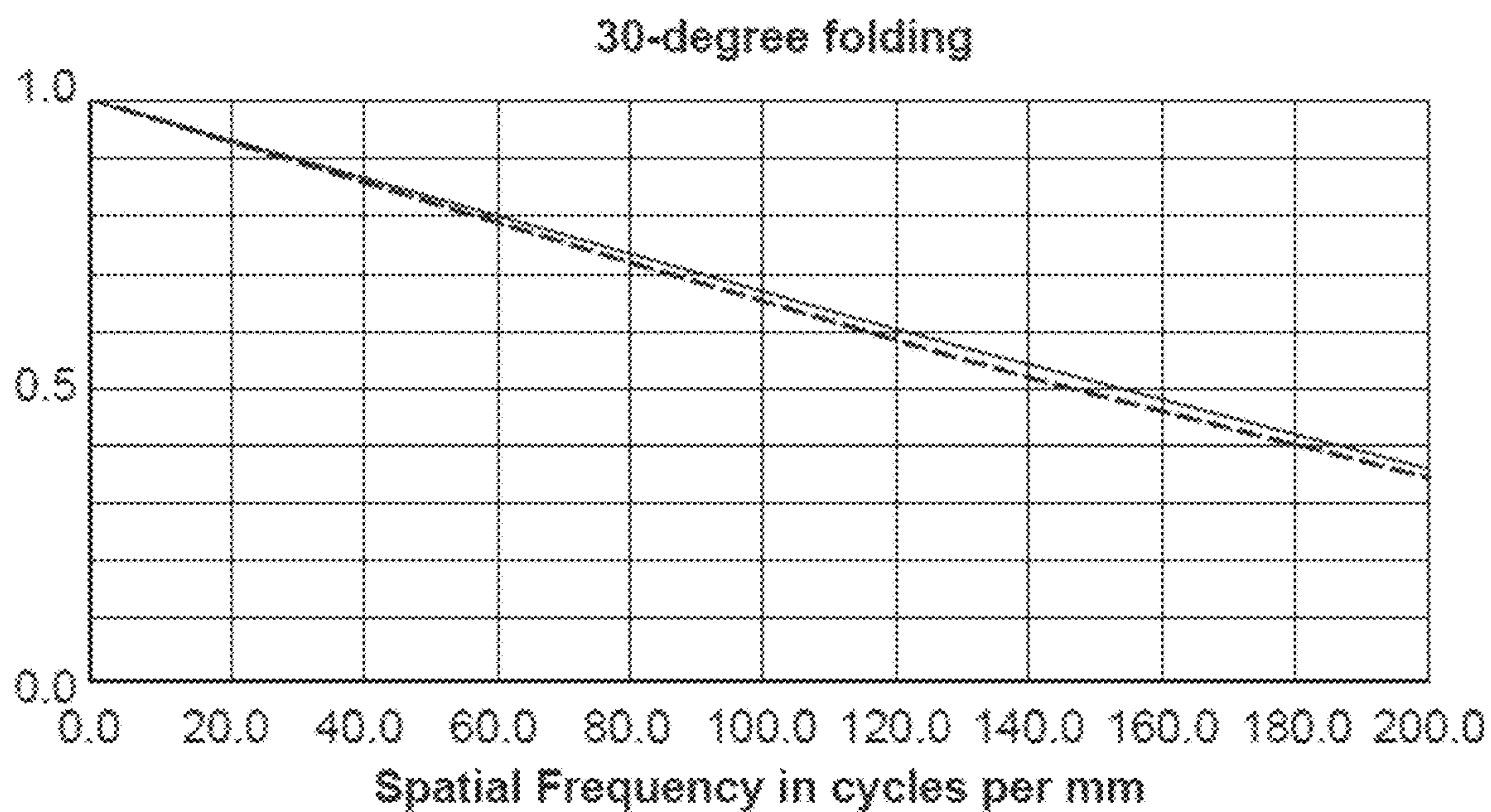
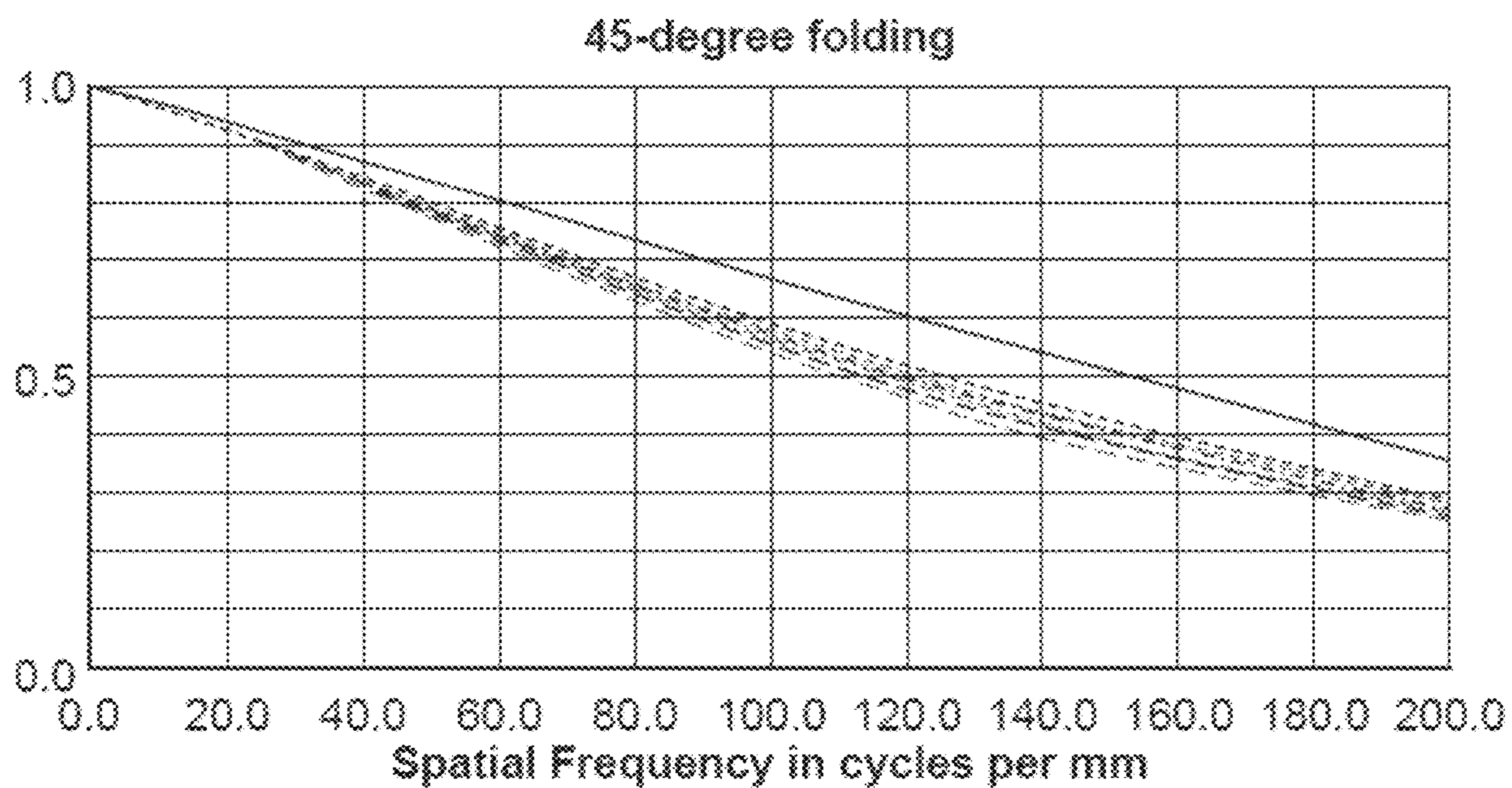


FIG. 9A

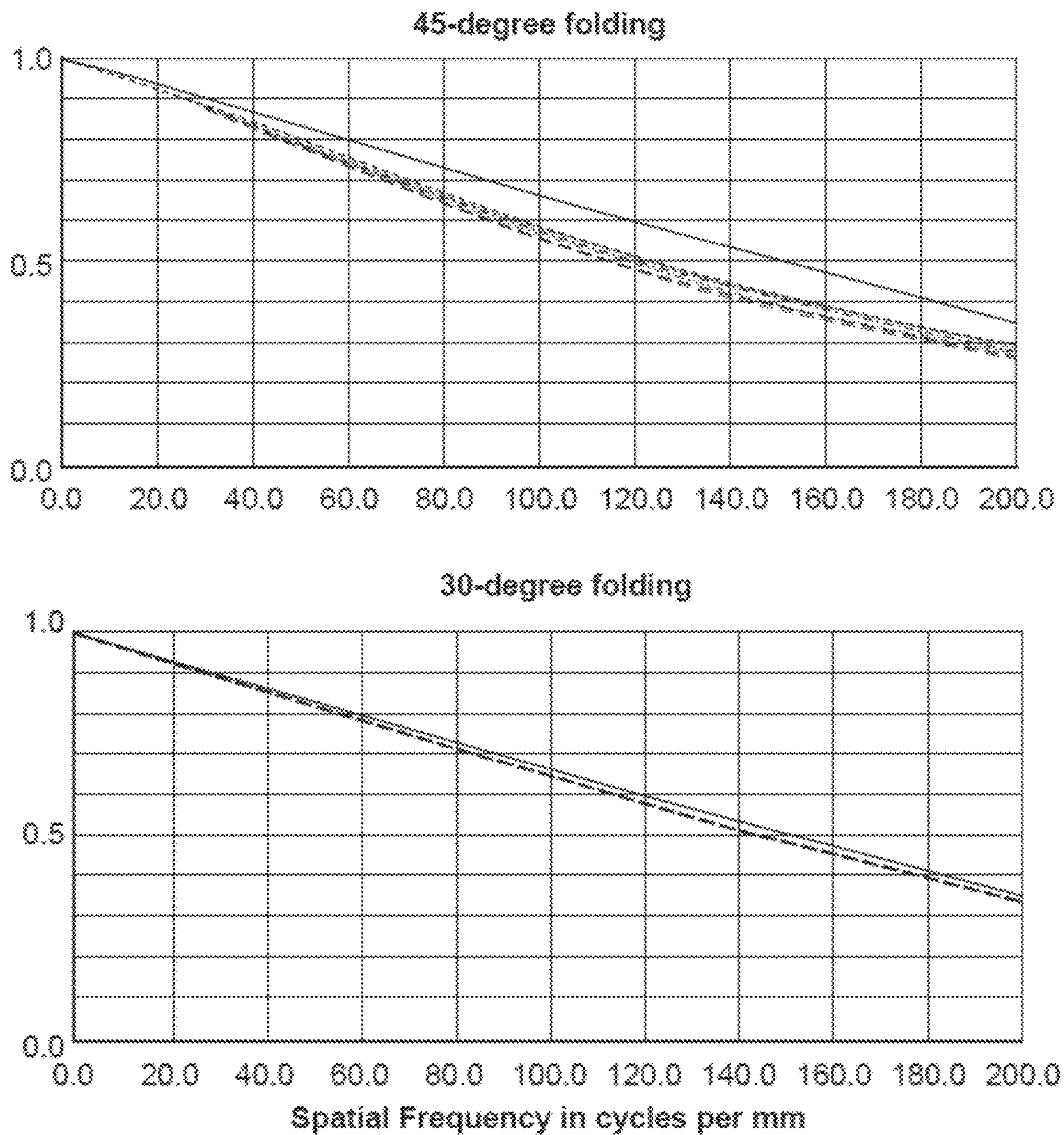


FIG. 9B

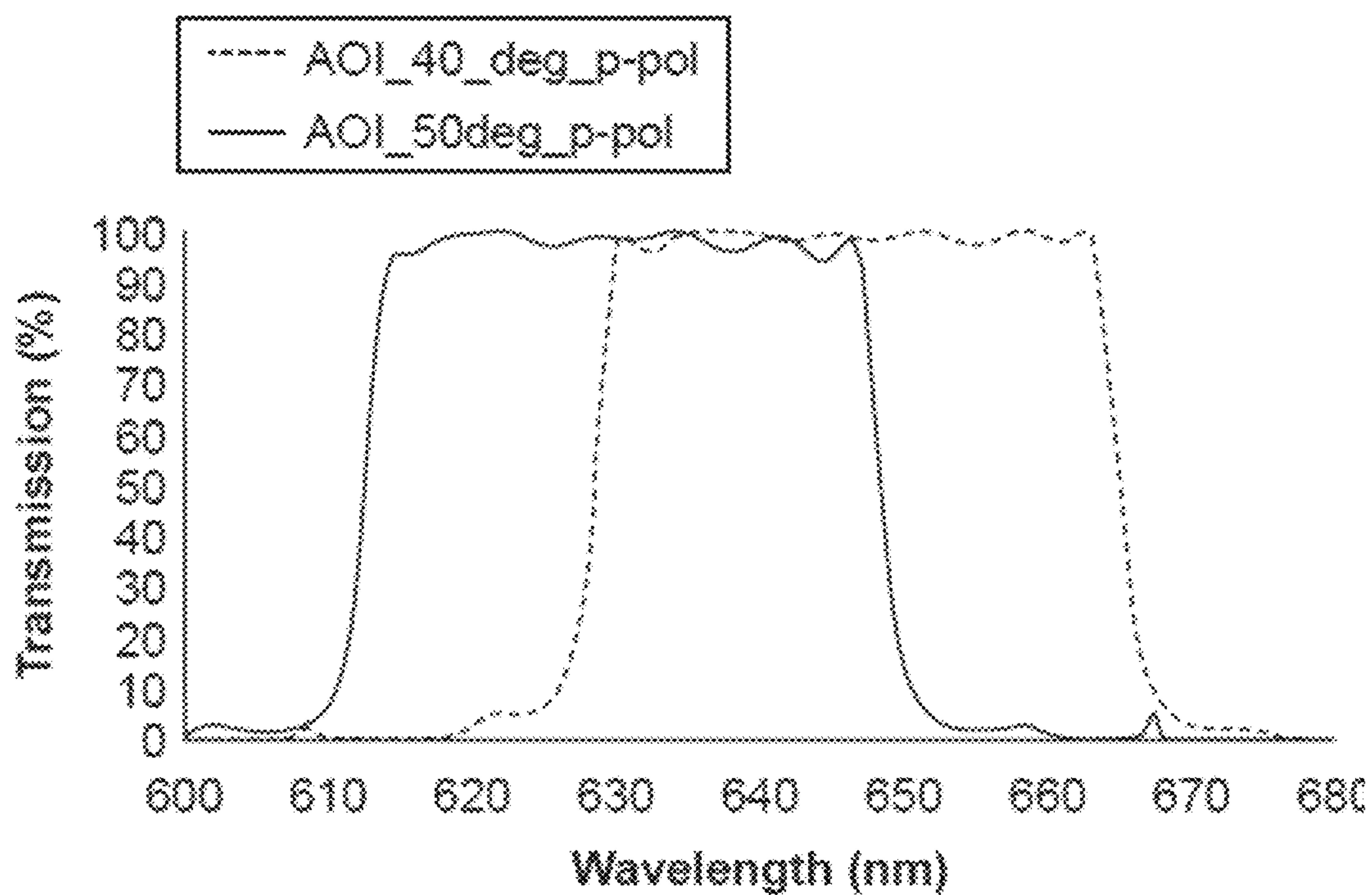


FIG. 10A

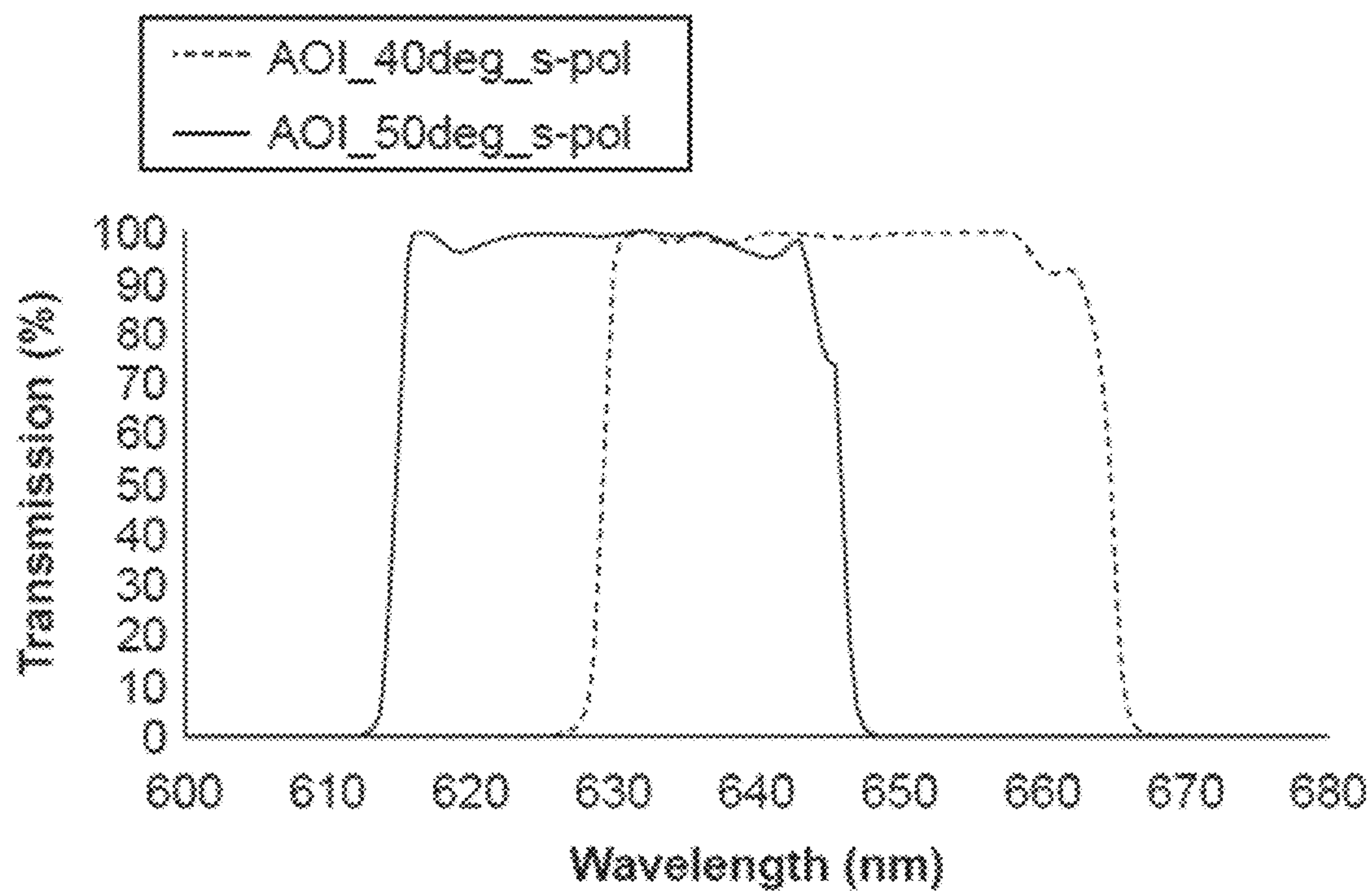


FIG. 10B

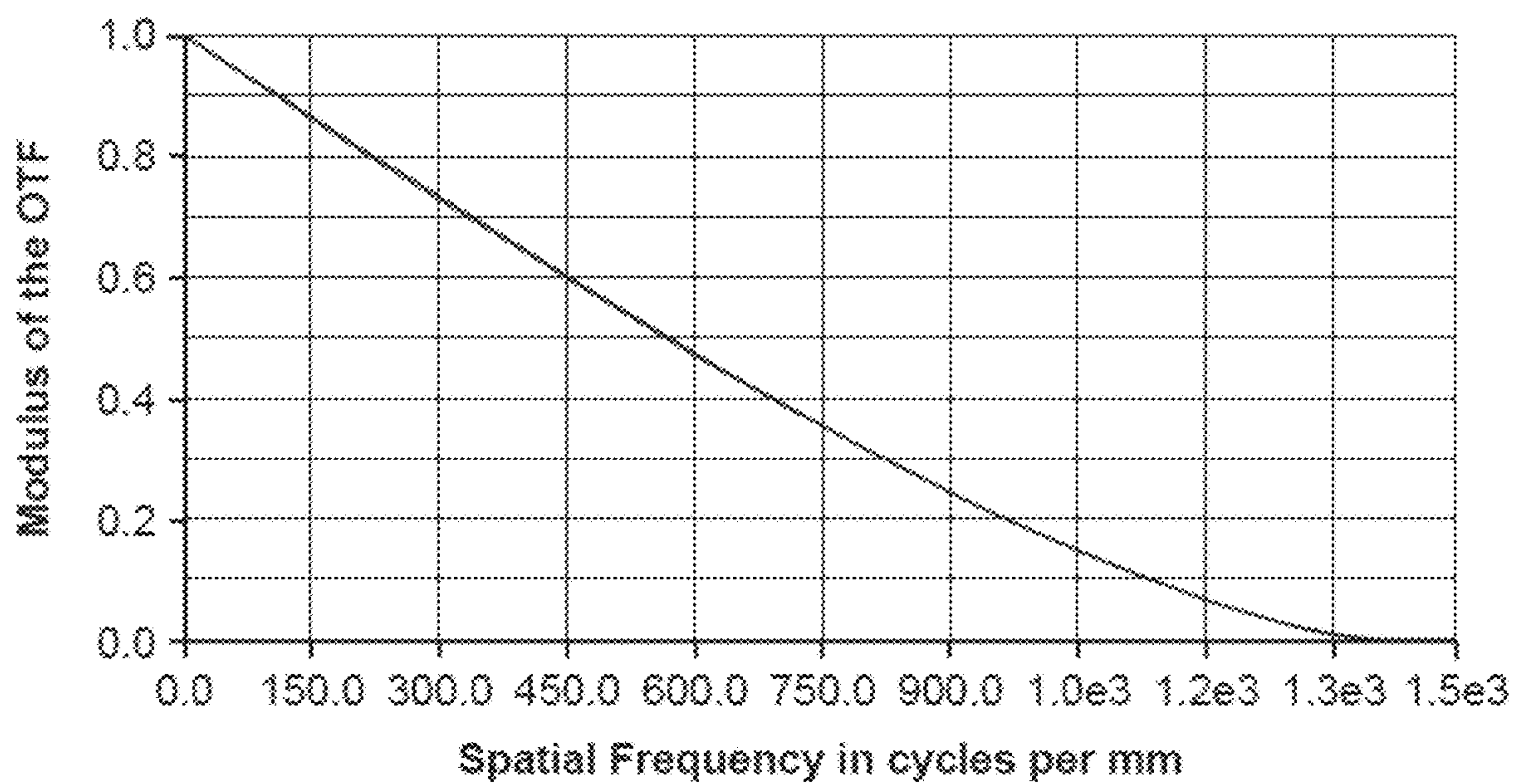


FIG. 11A

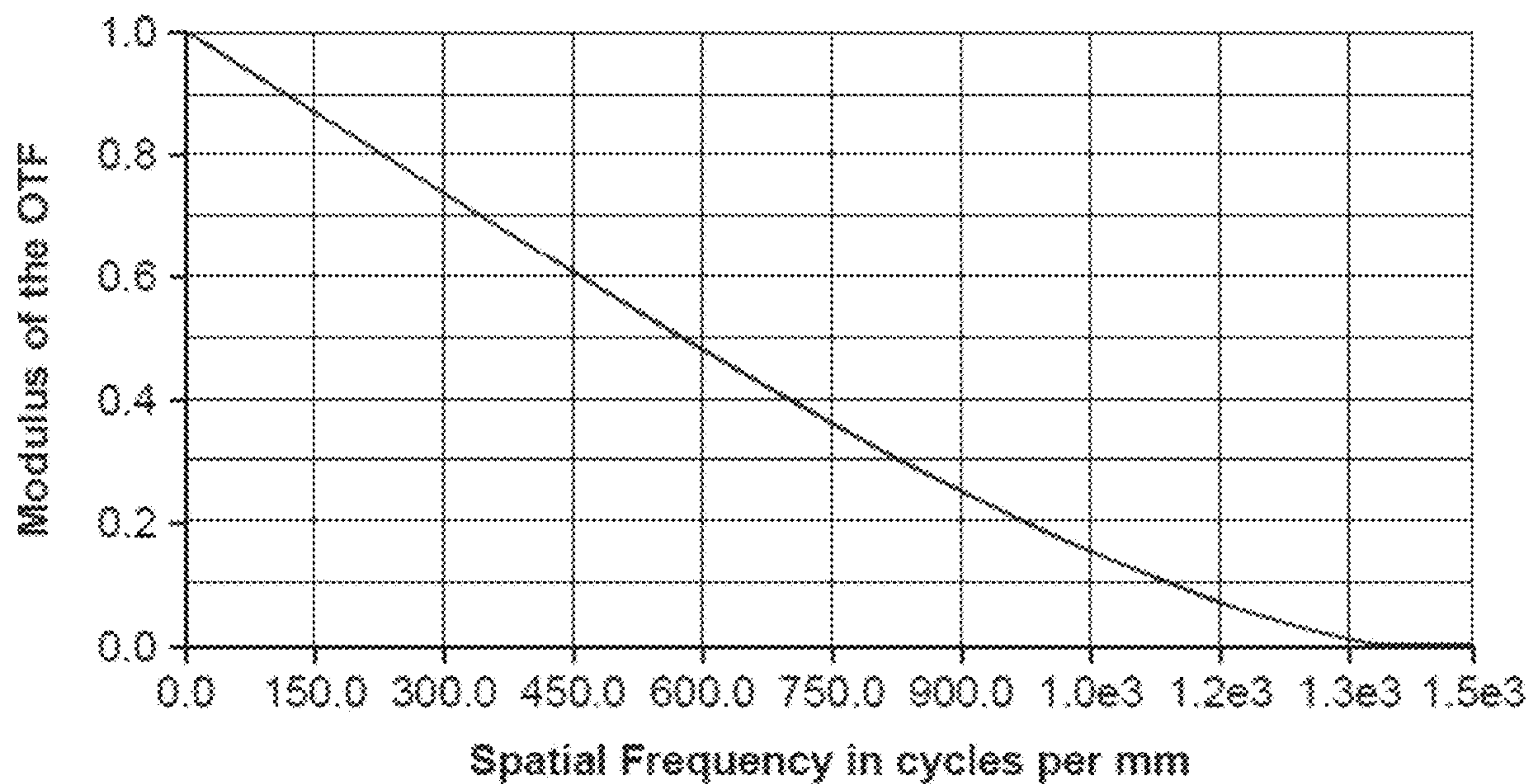


FIG. 11B

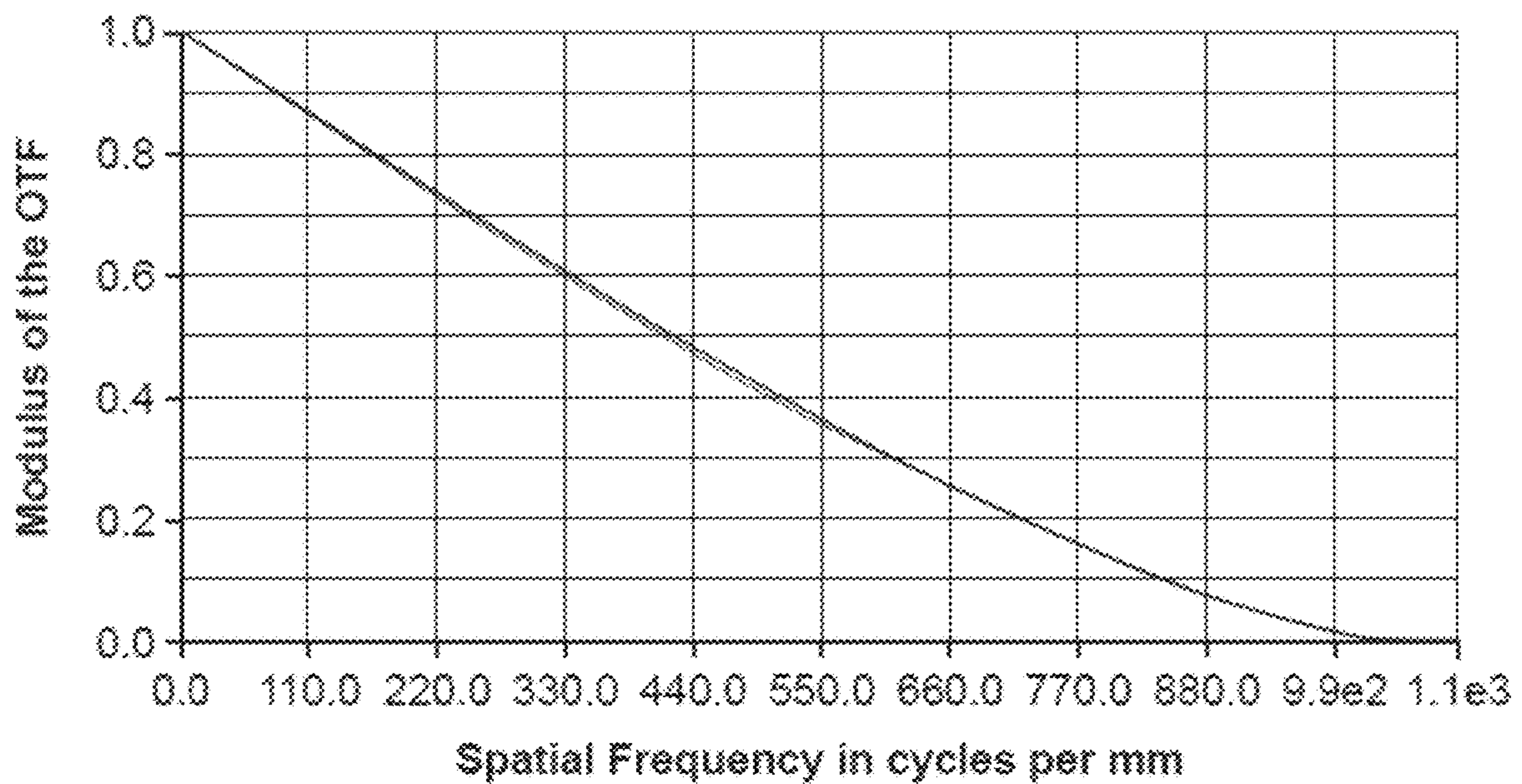


FIG. 12A

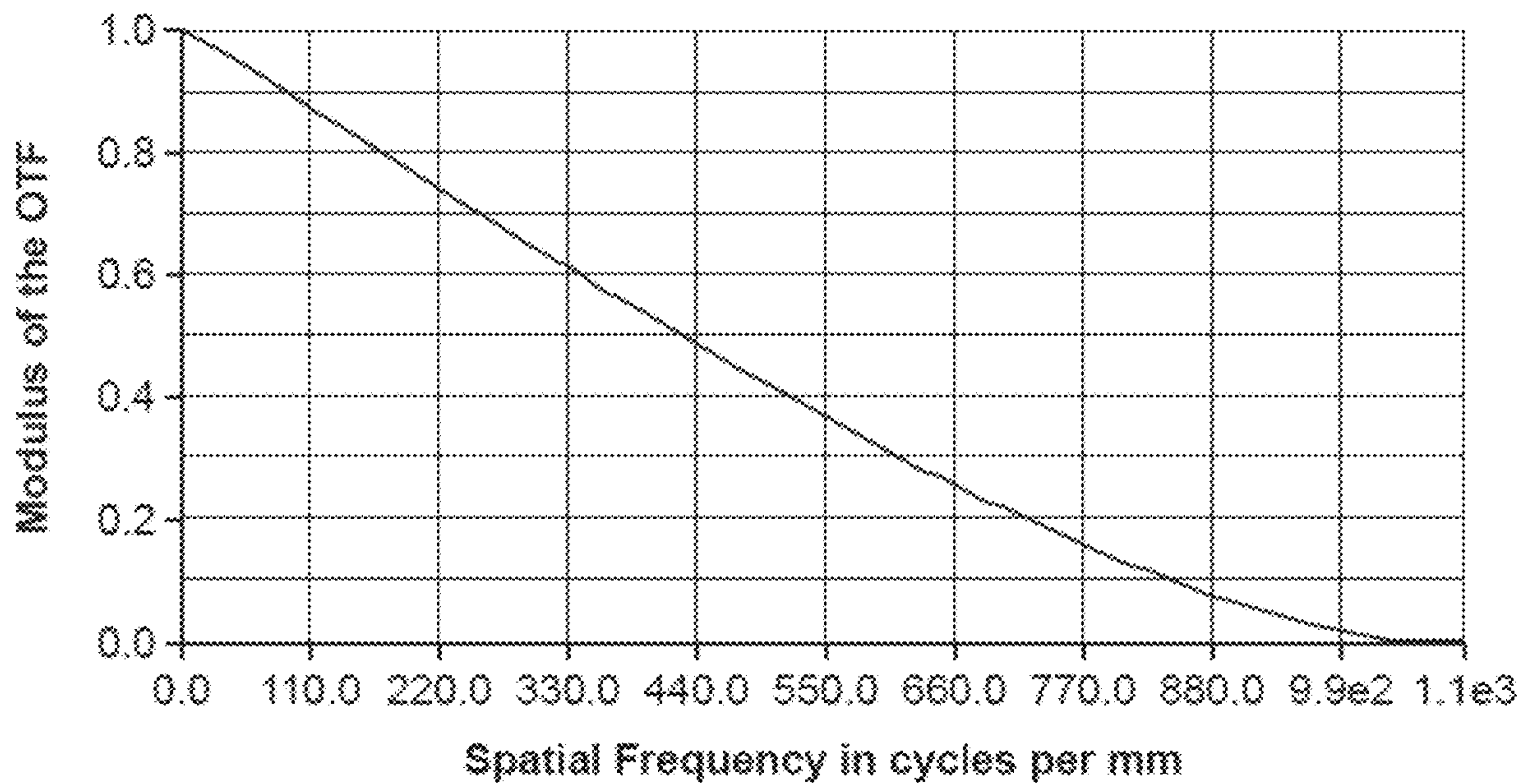


FIG. 12B

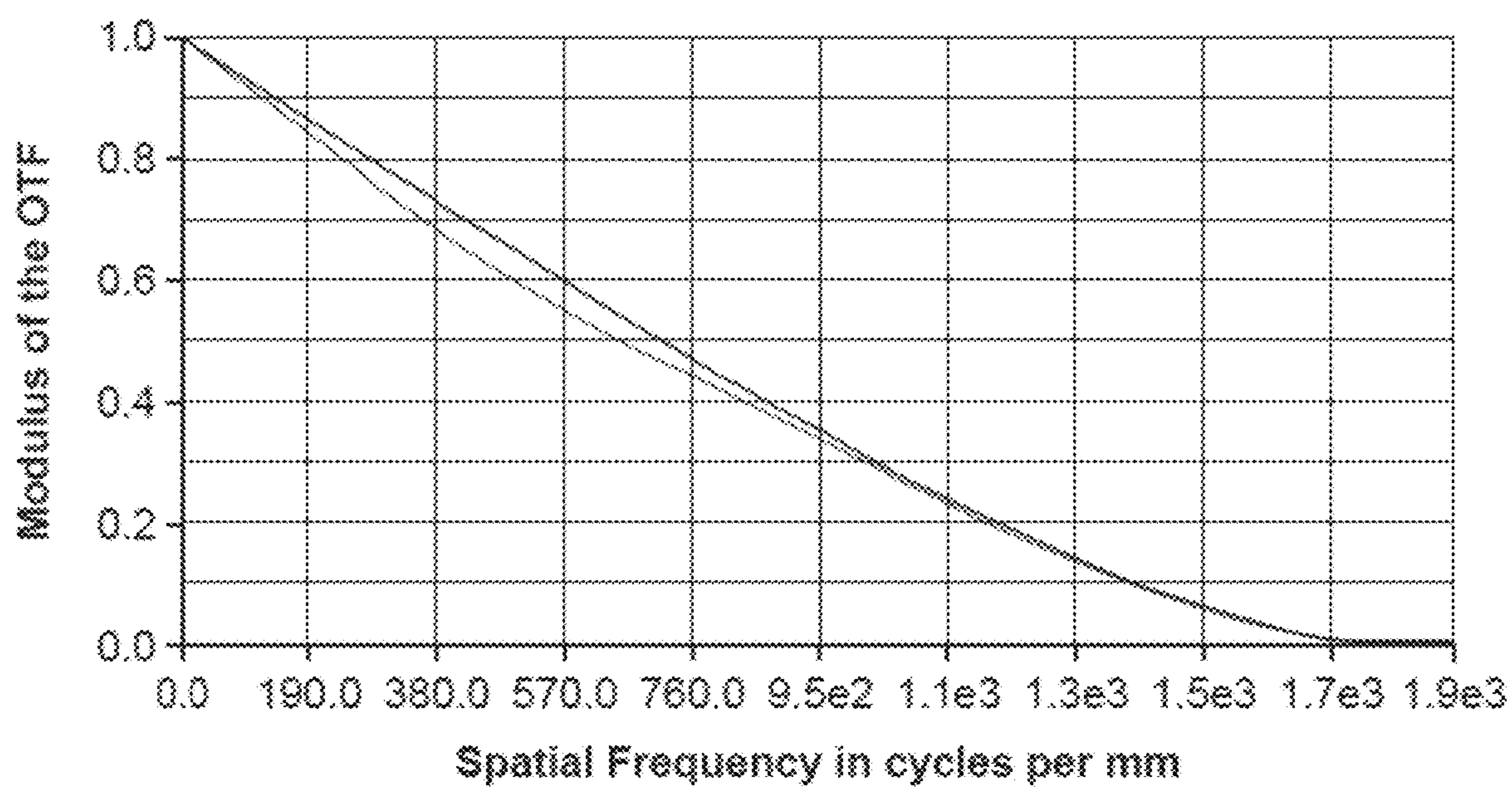


FIG. 13A

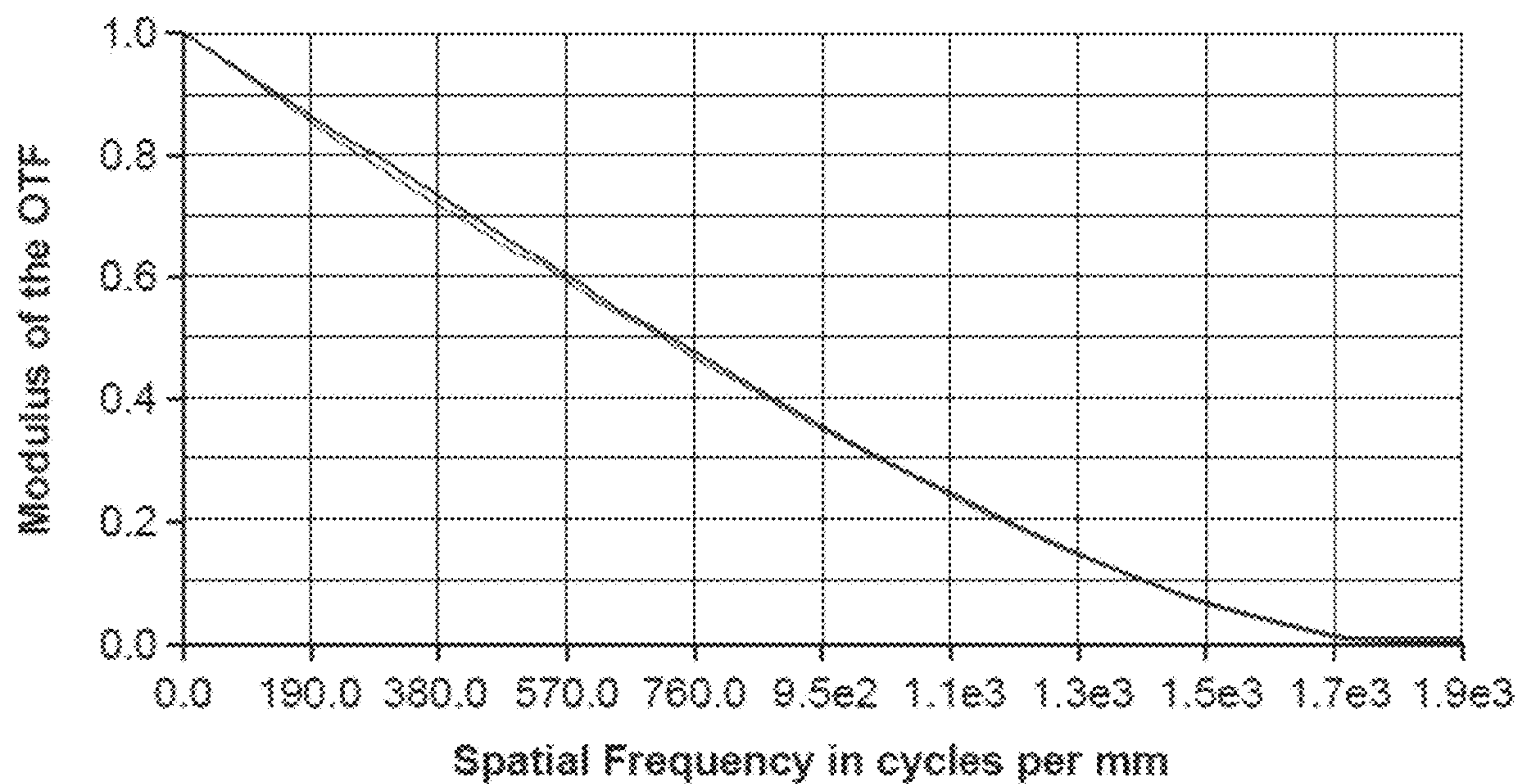


FIG. 13B

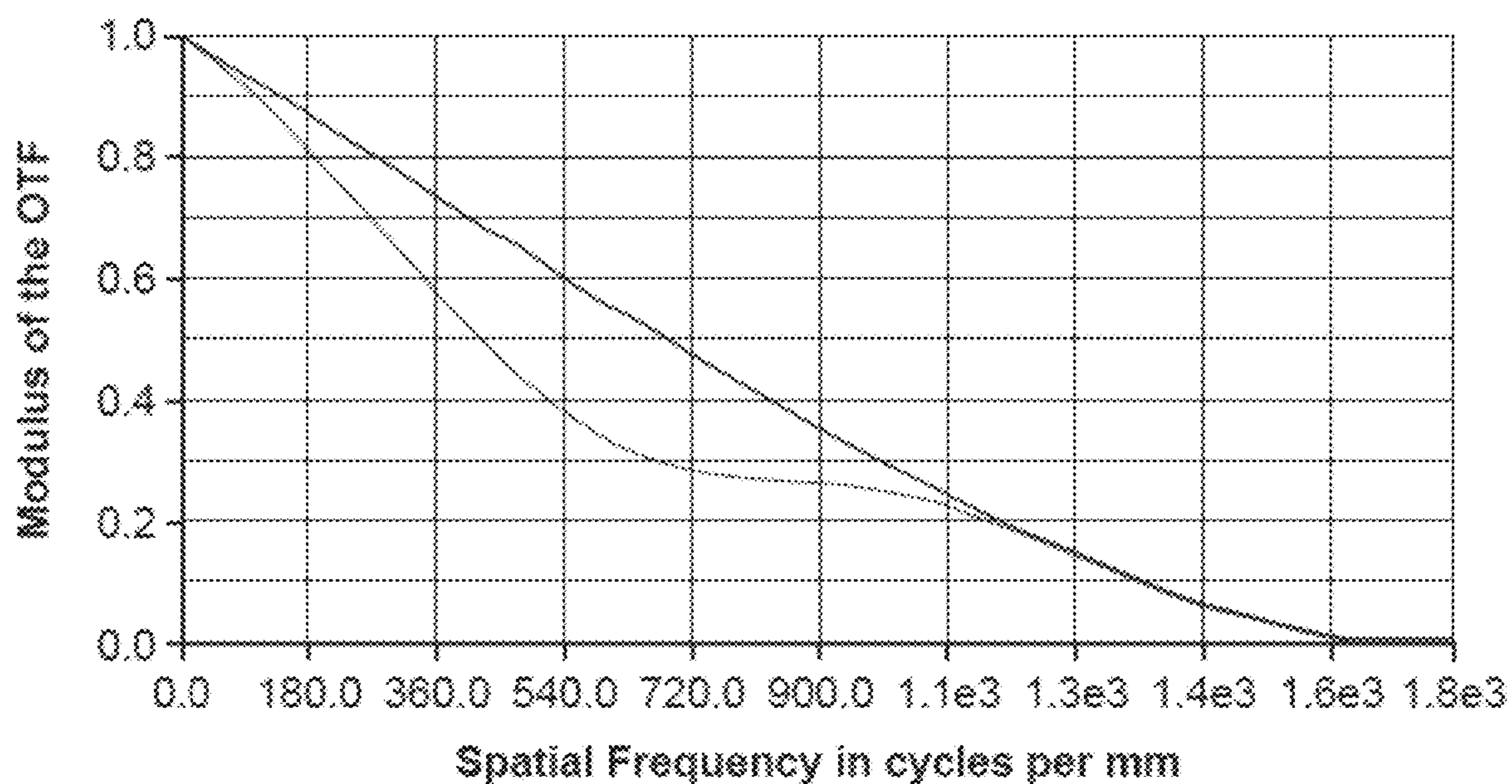


FIG. 14A

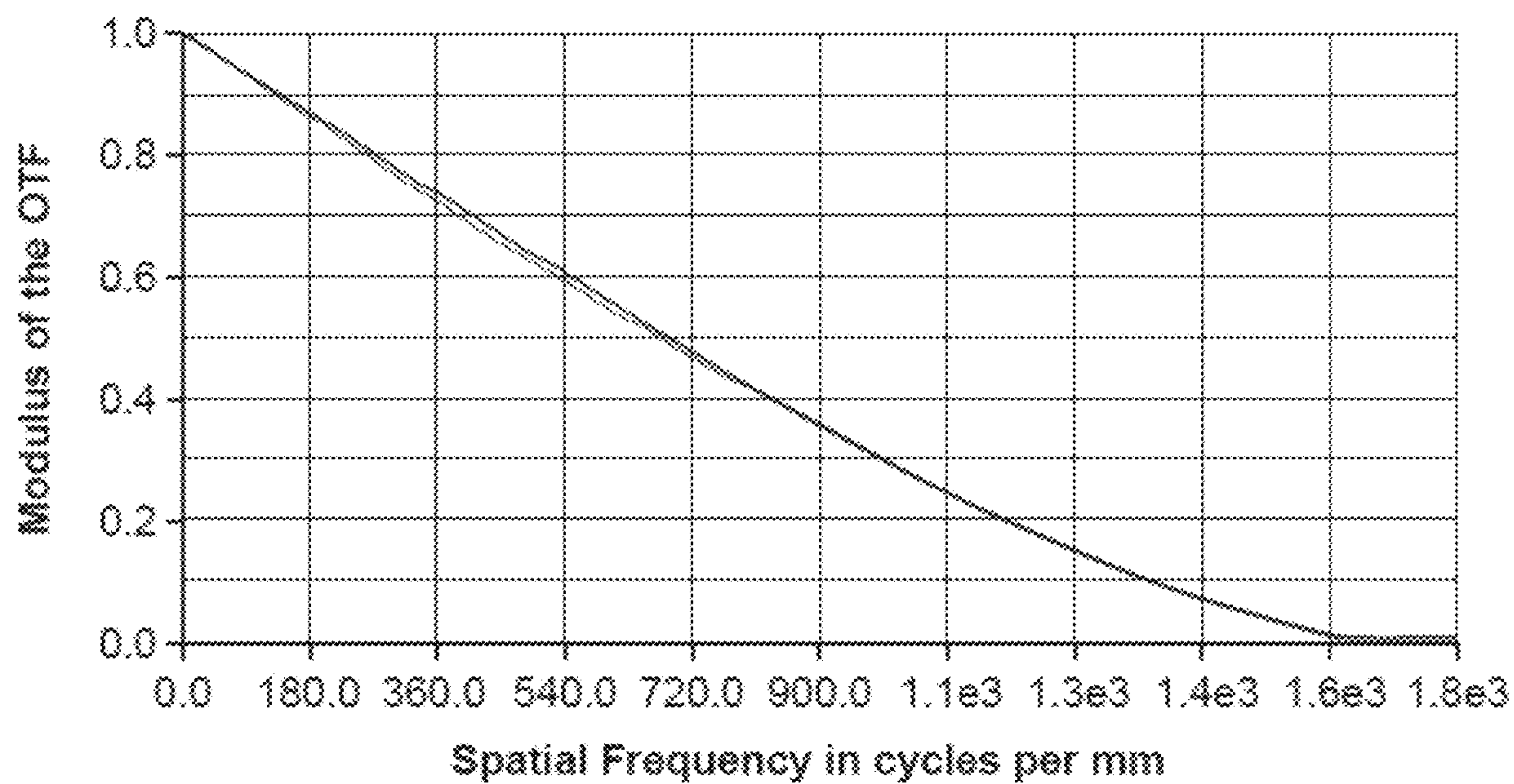


FIG. 14B

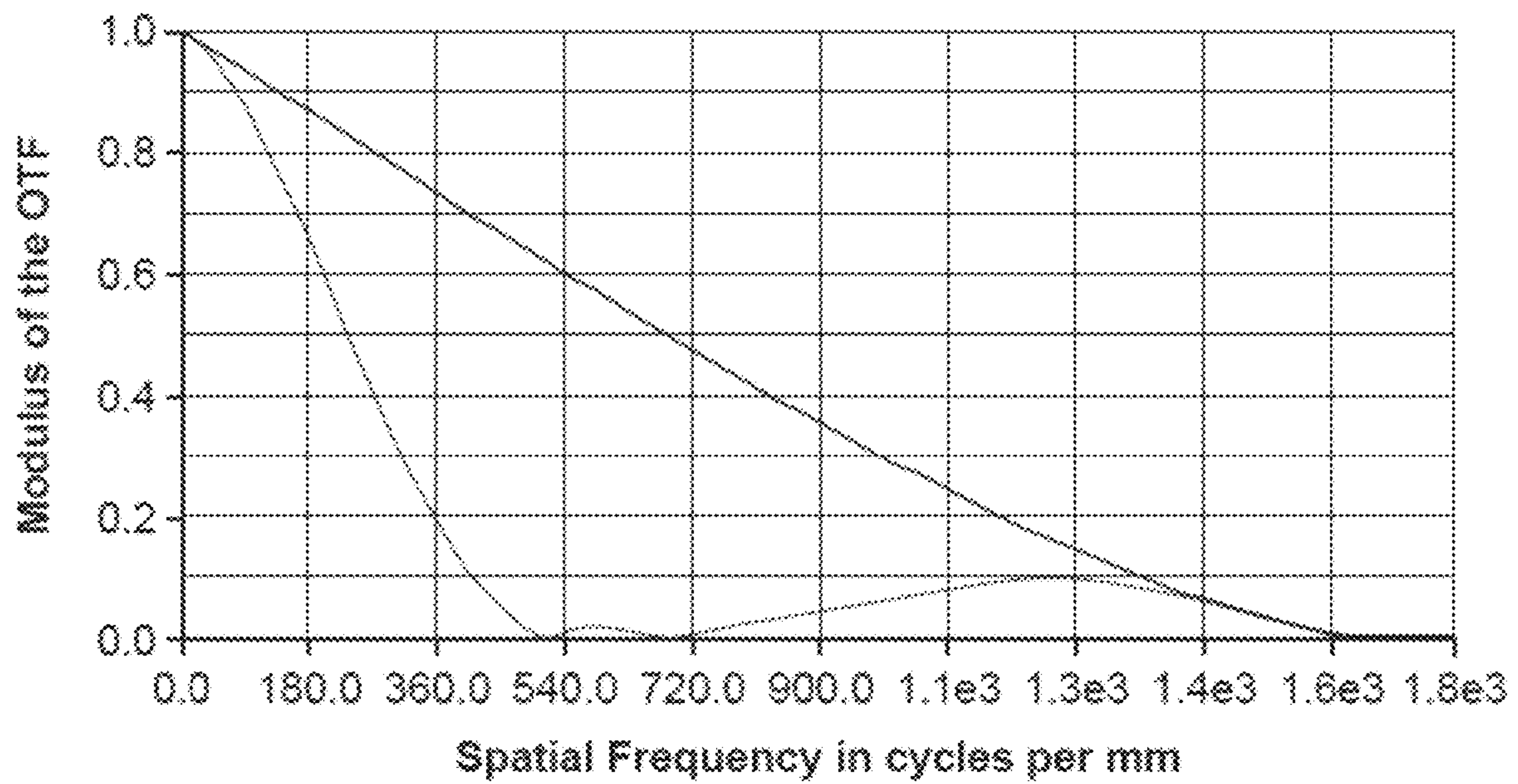


FIG. 15A

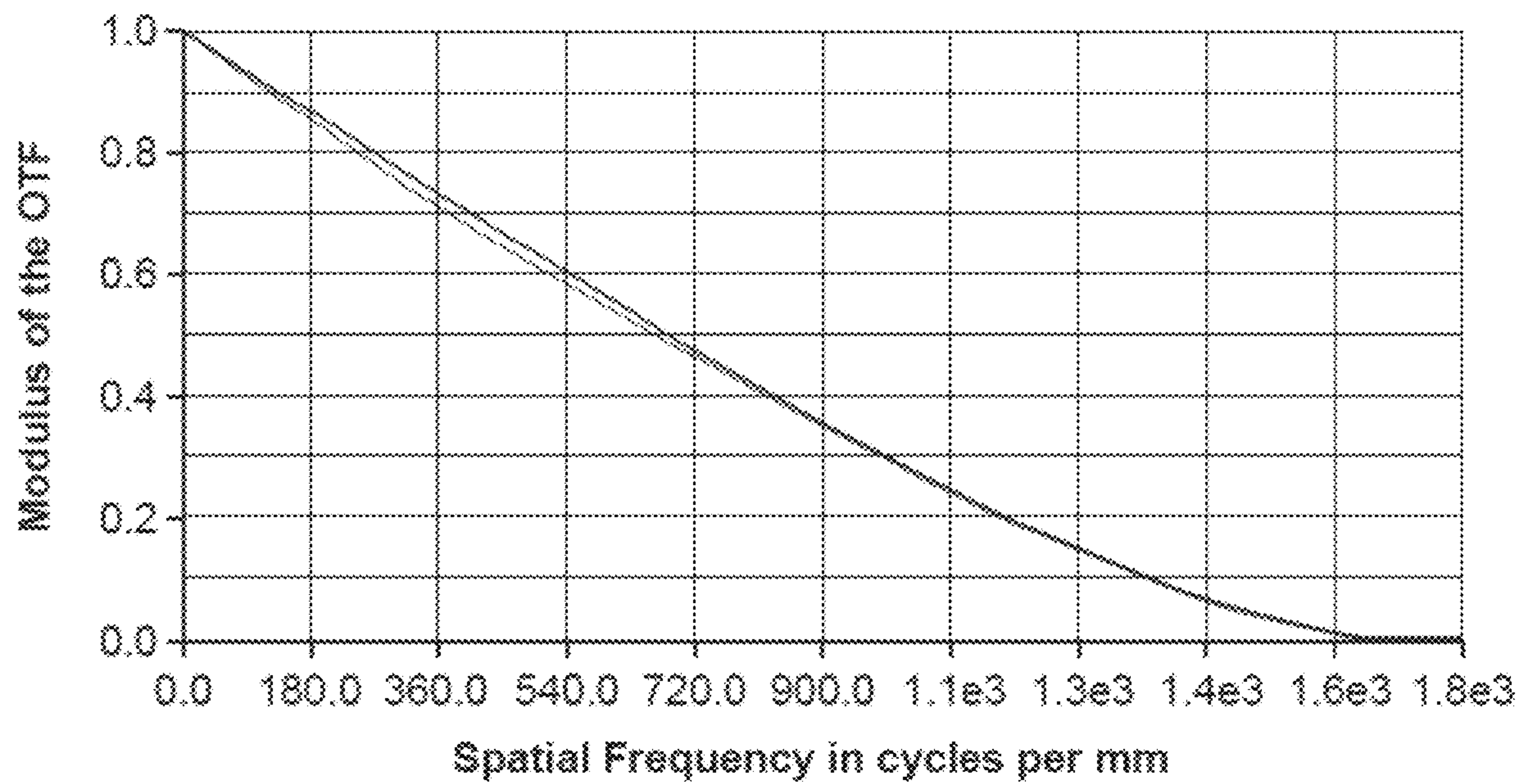


FIG. 15B

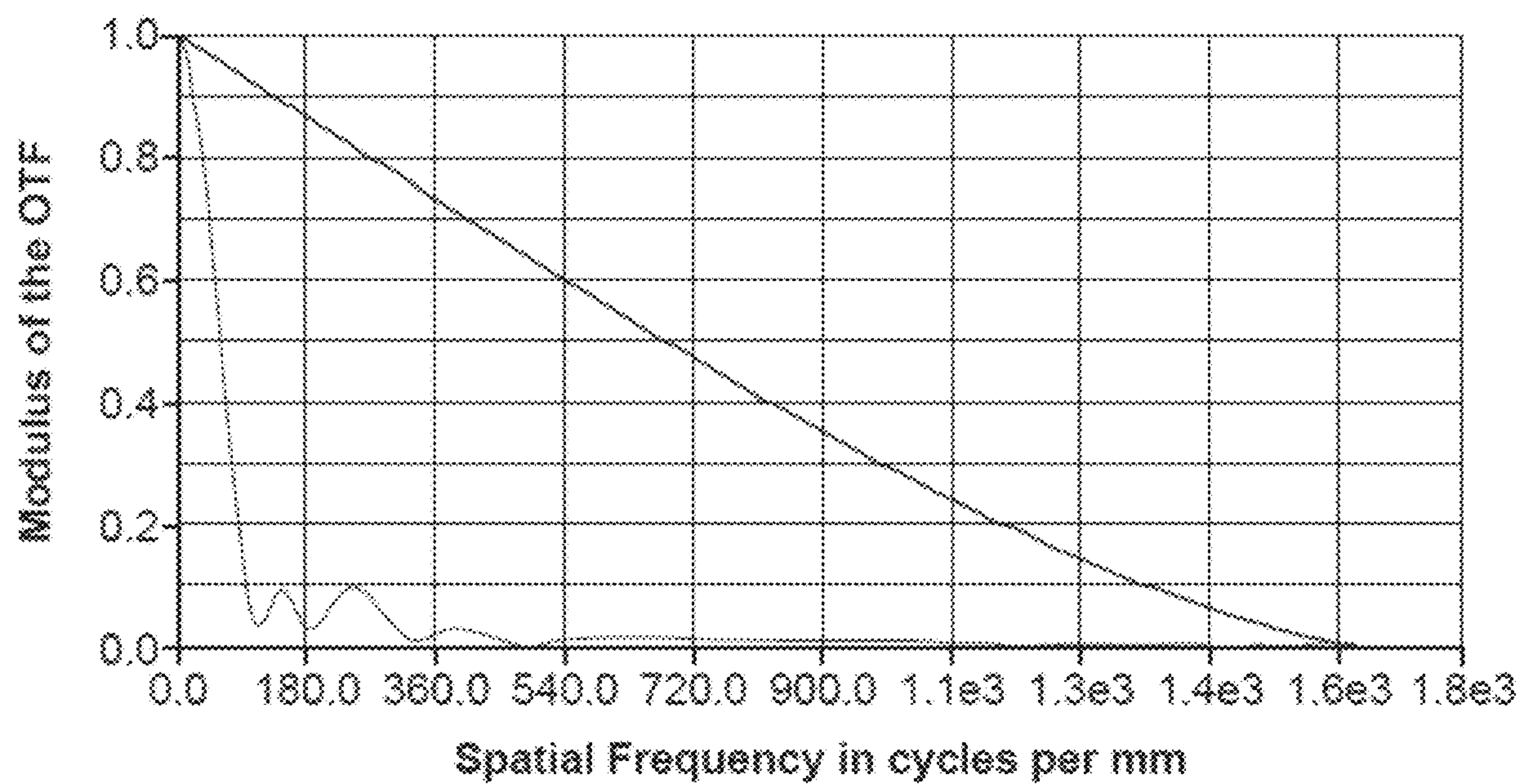


FIG. 16A

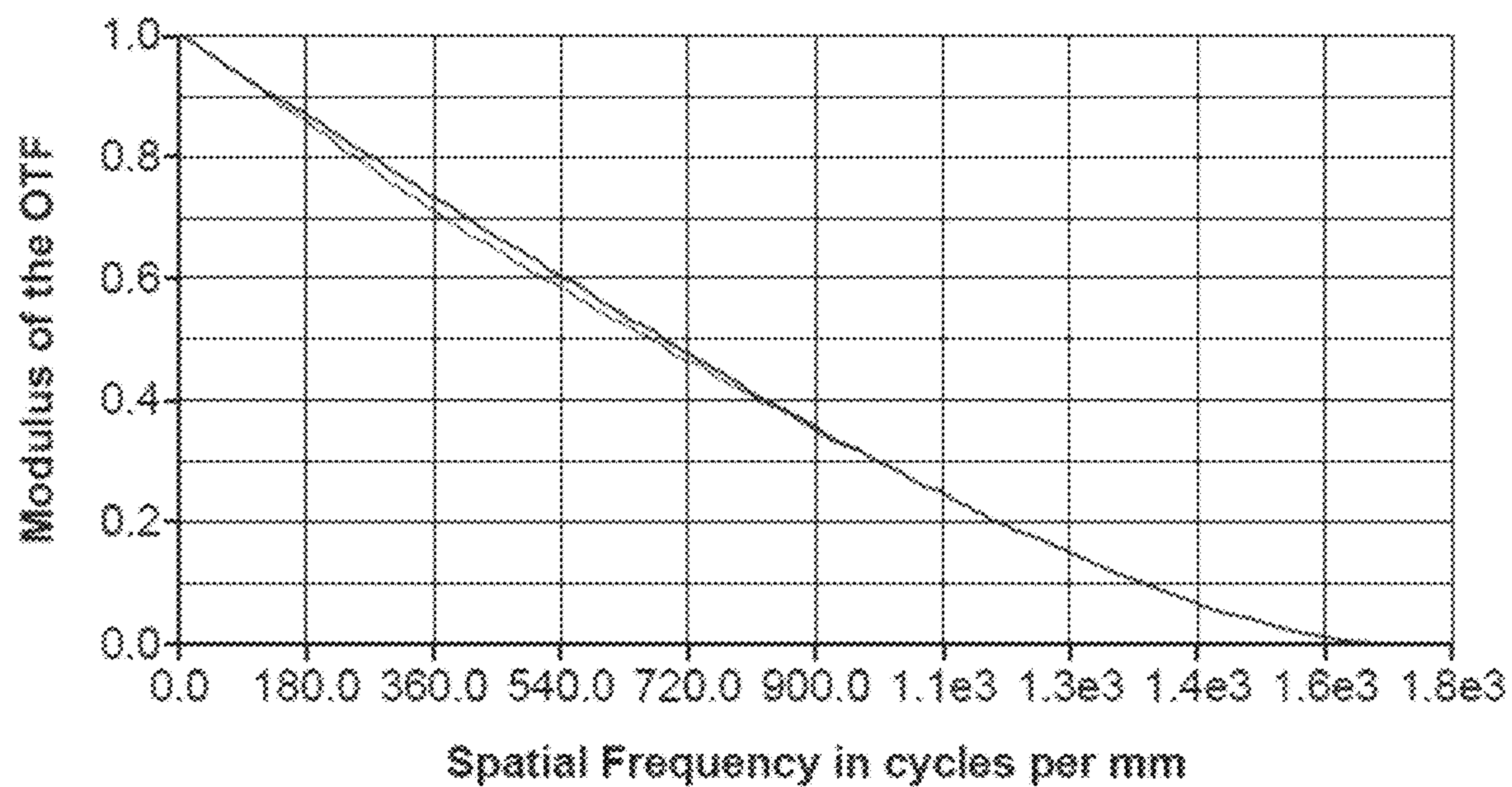


FIG. 16B

Strehl ratio change with thickness, field=0mm, EFL_objective=20mm, Mag=5

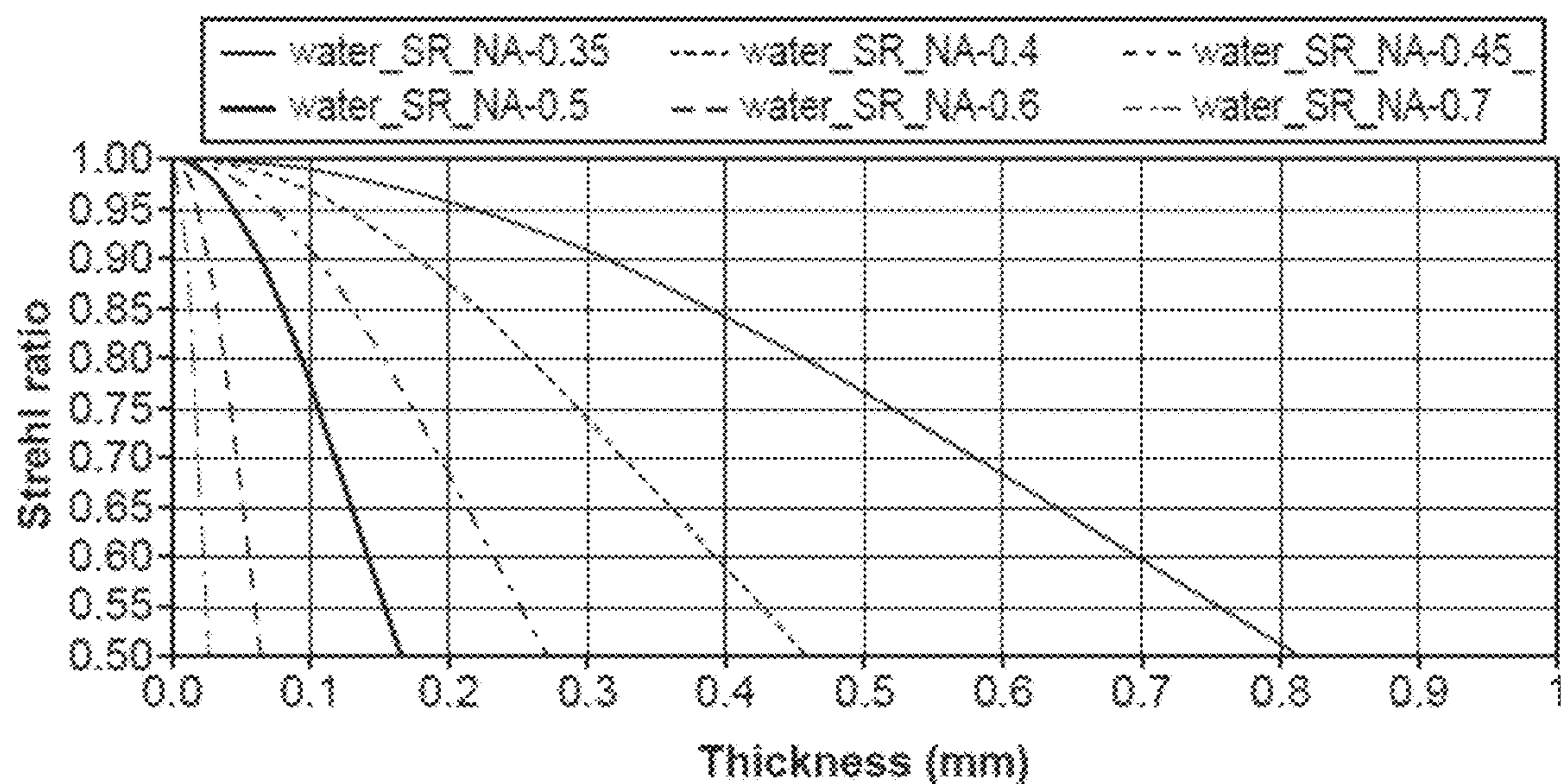


FIG. 17A

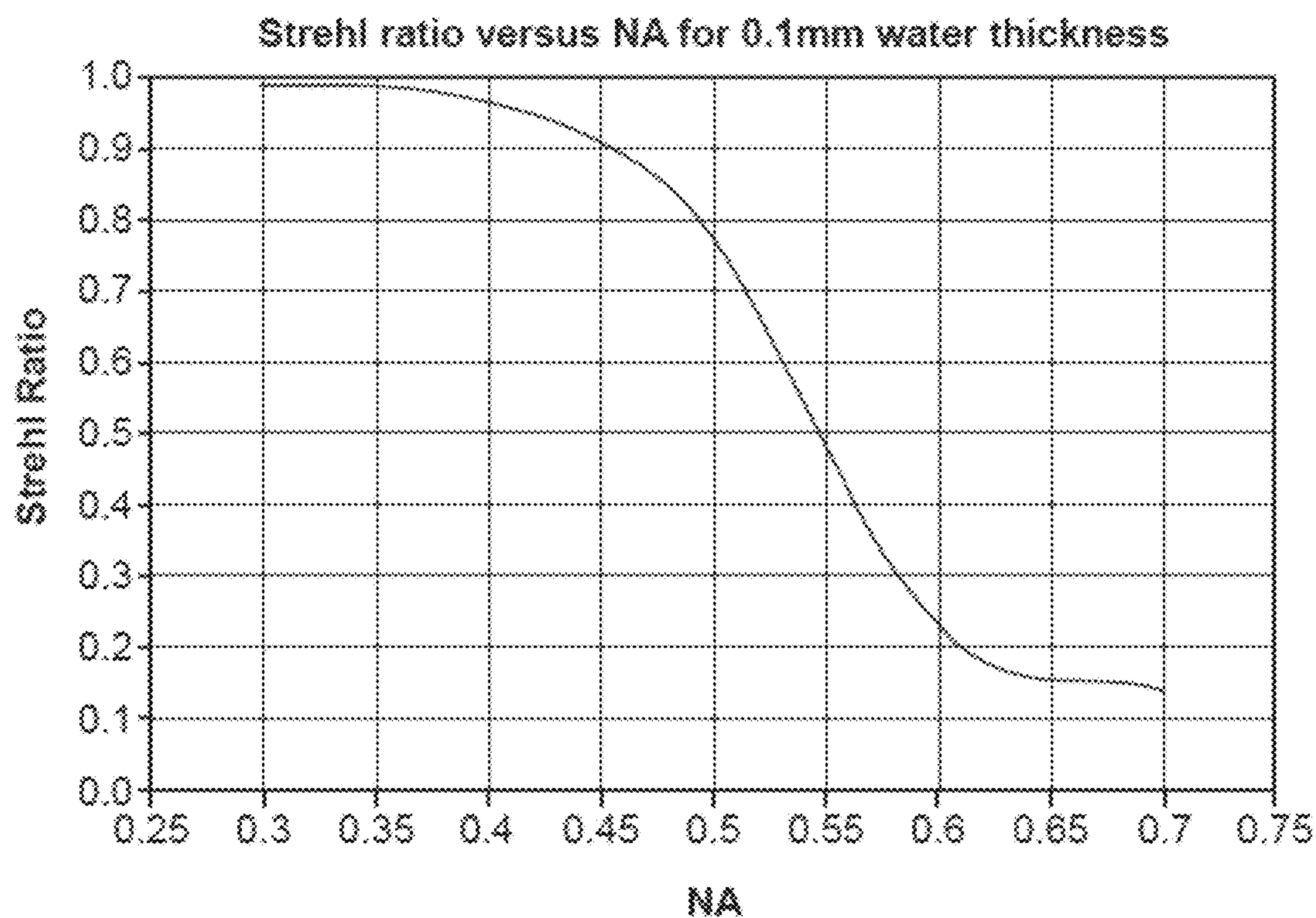


FIG. 17B

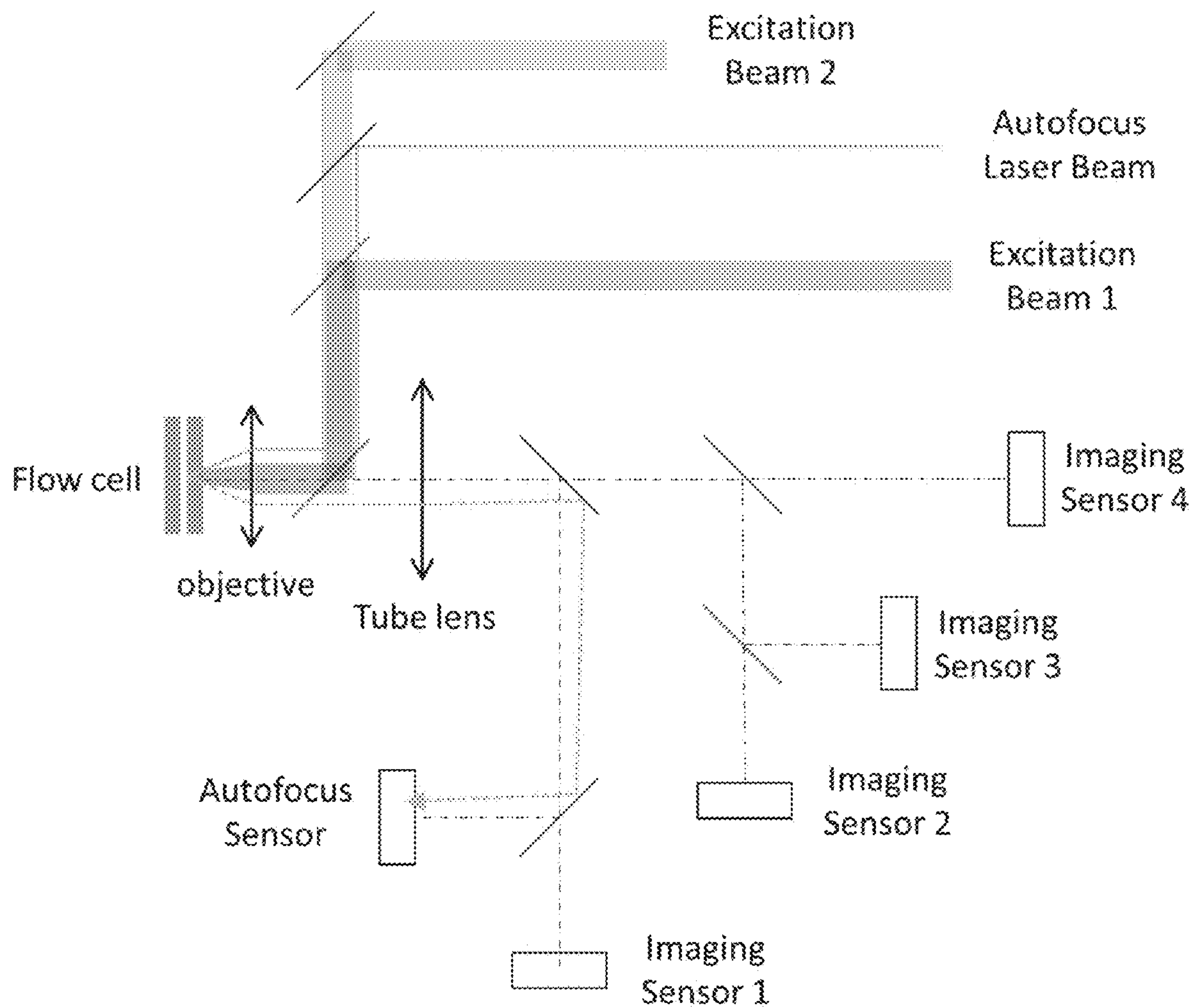


FIG. 18

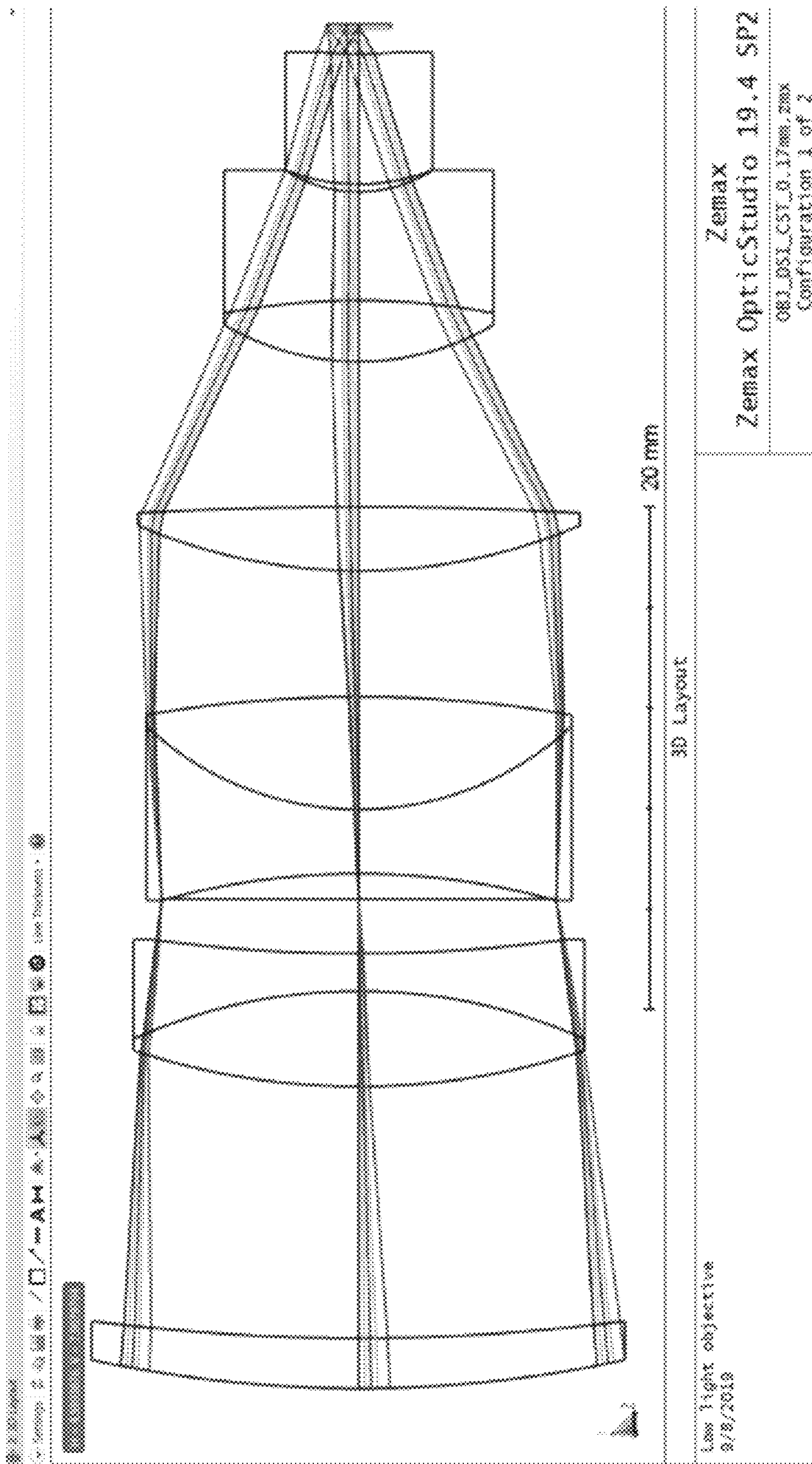
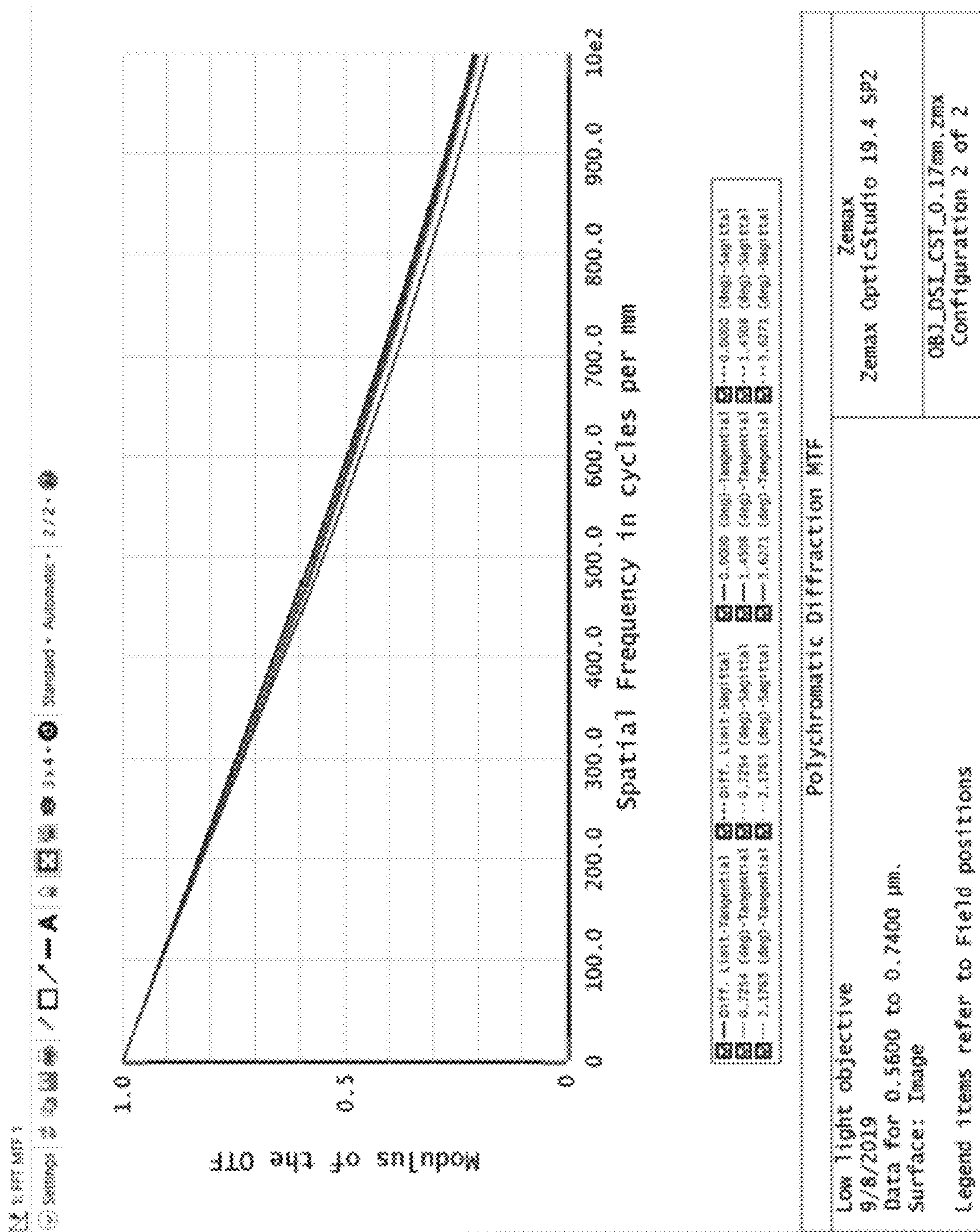


FIG. 19



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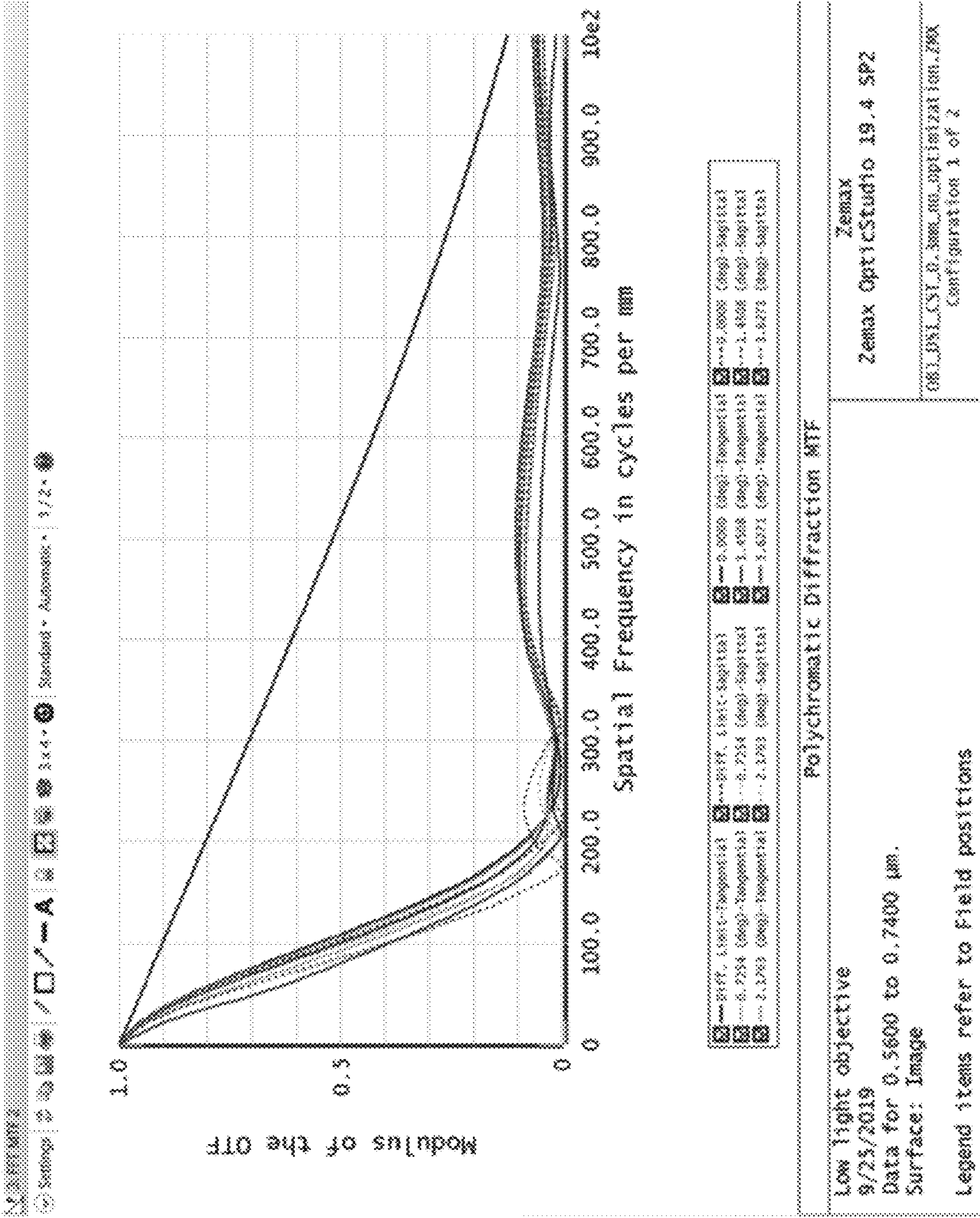


FIG. 23



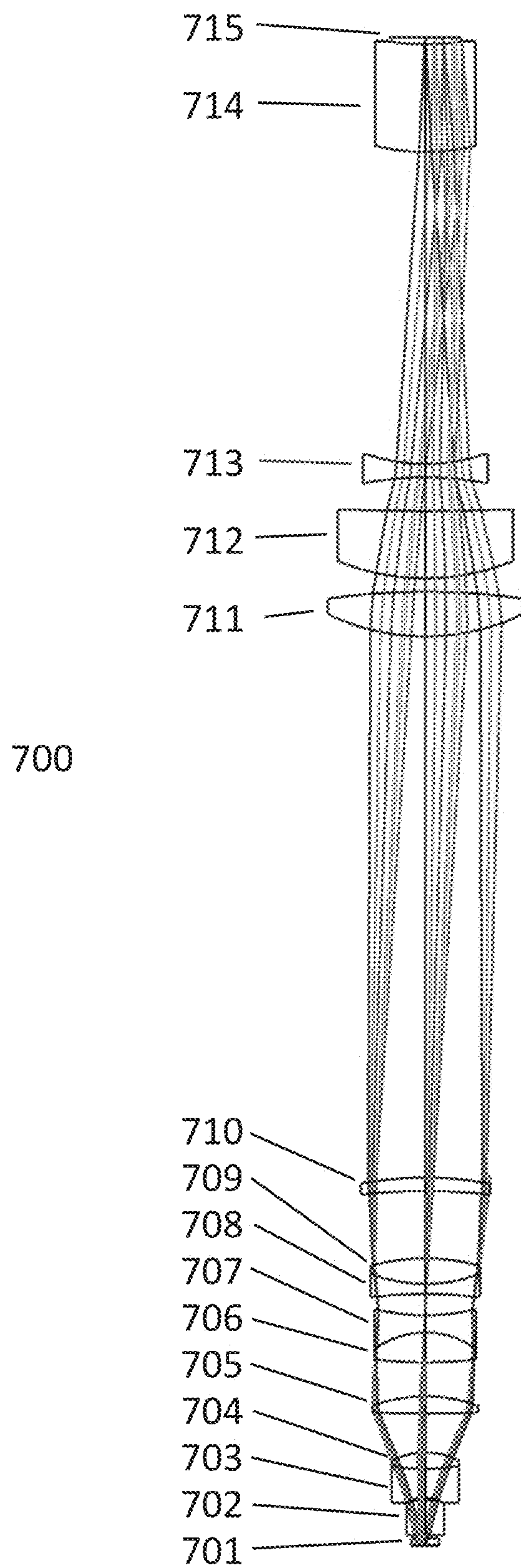


FIG. 25





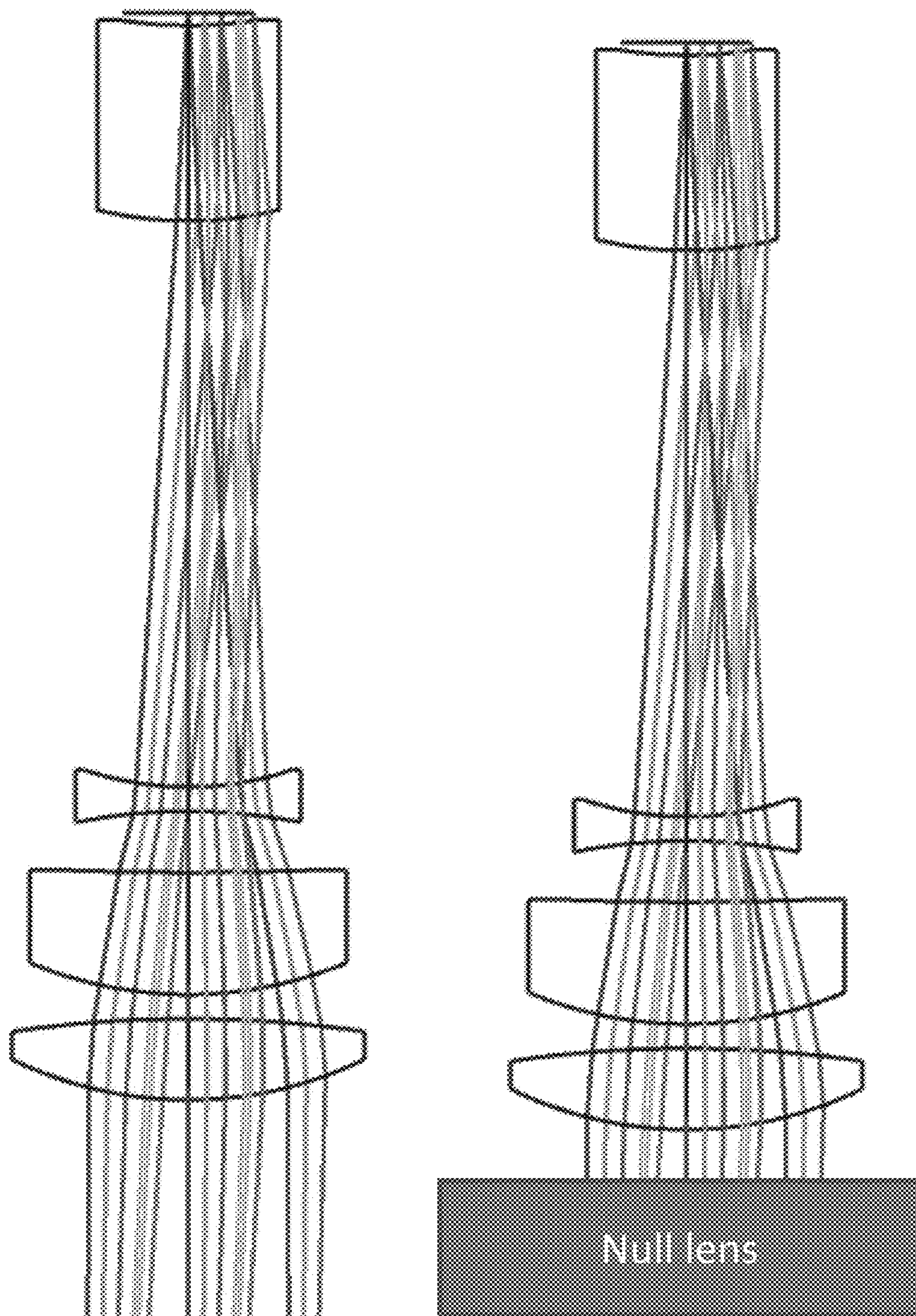


FIG. 28

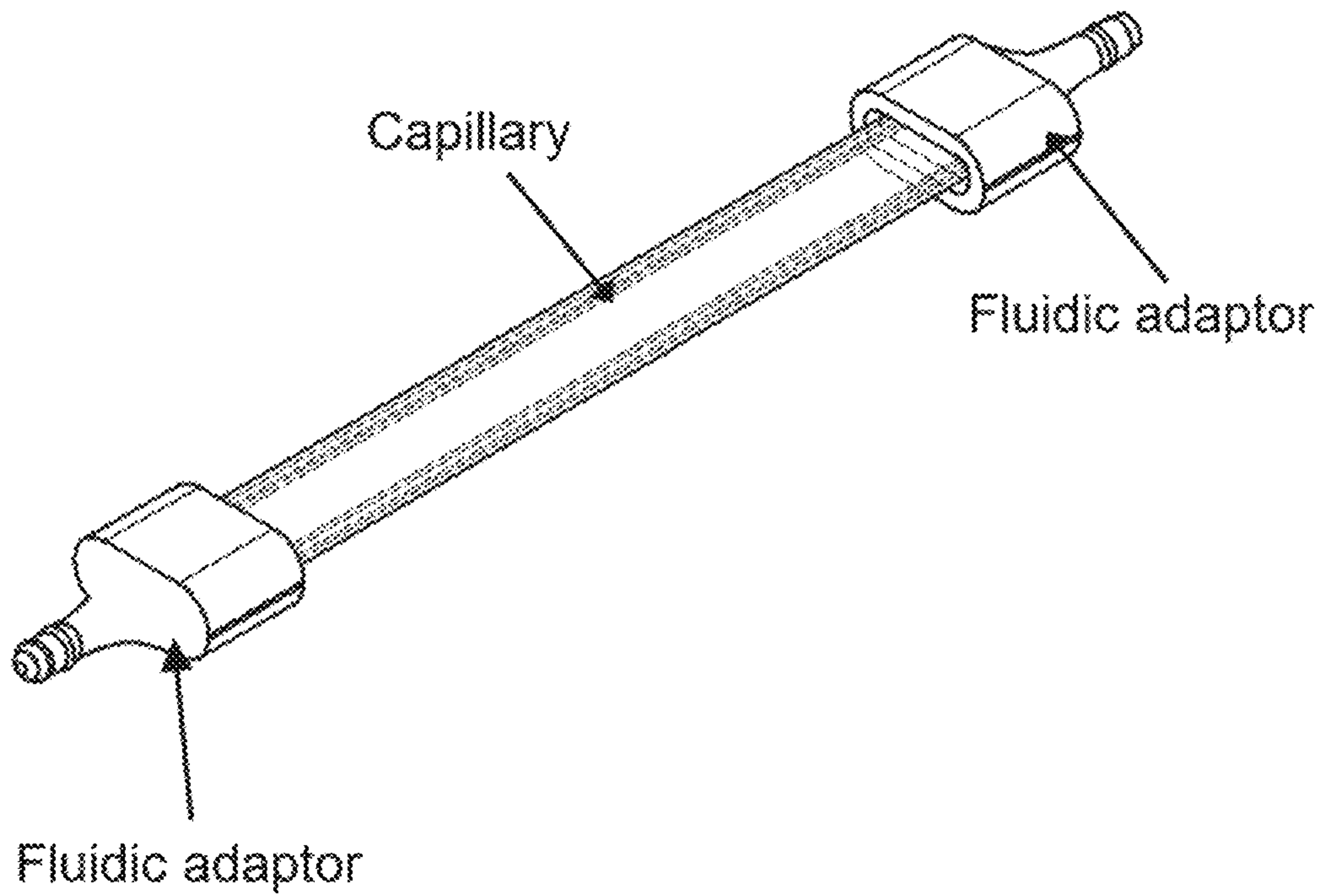


FIG. 29

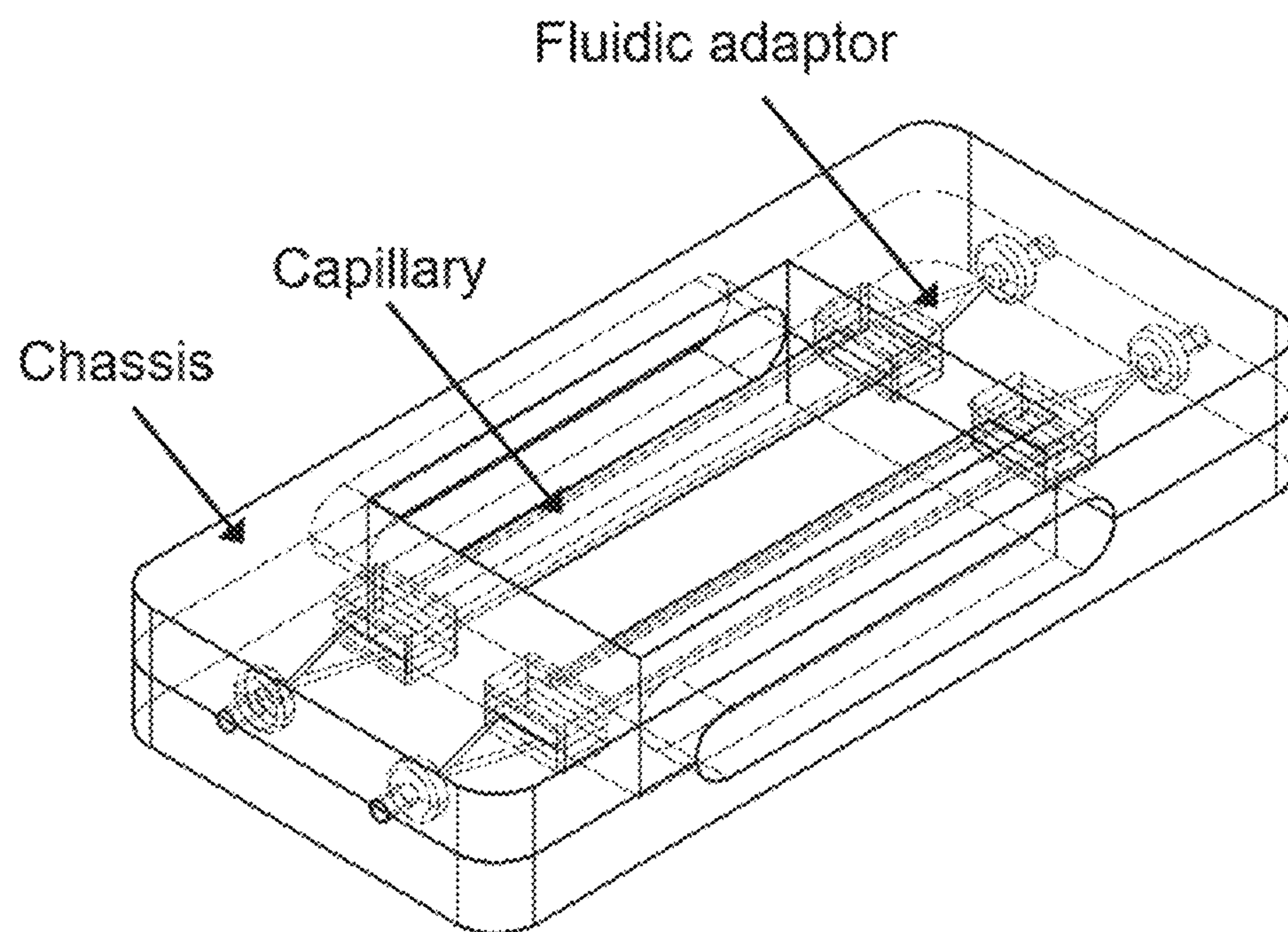


FIG. 30

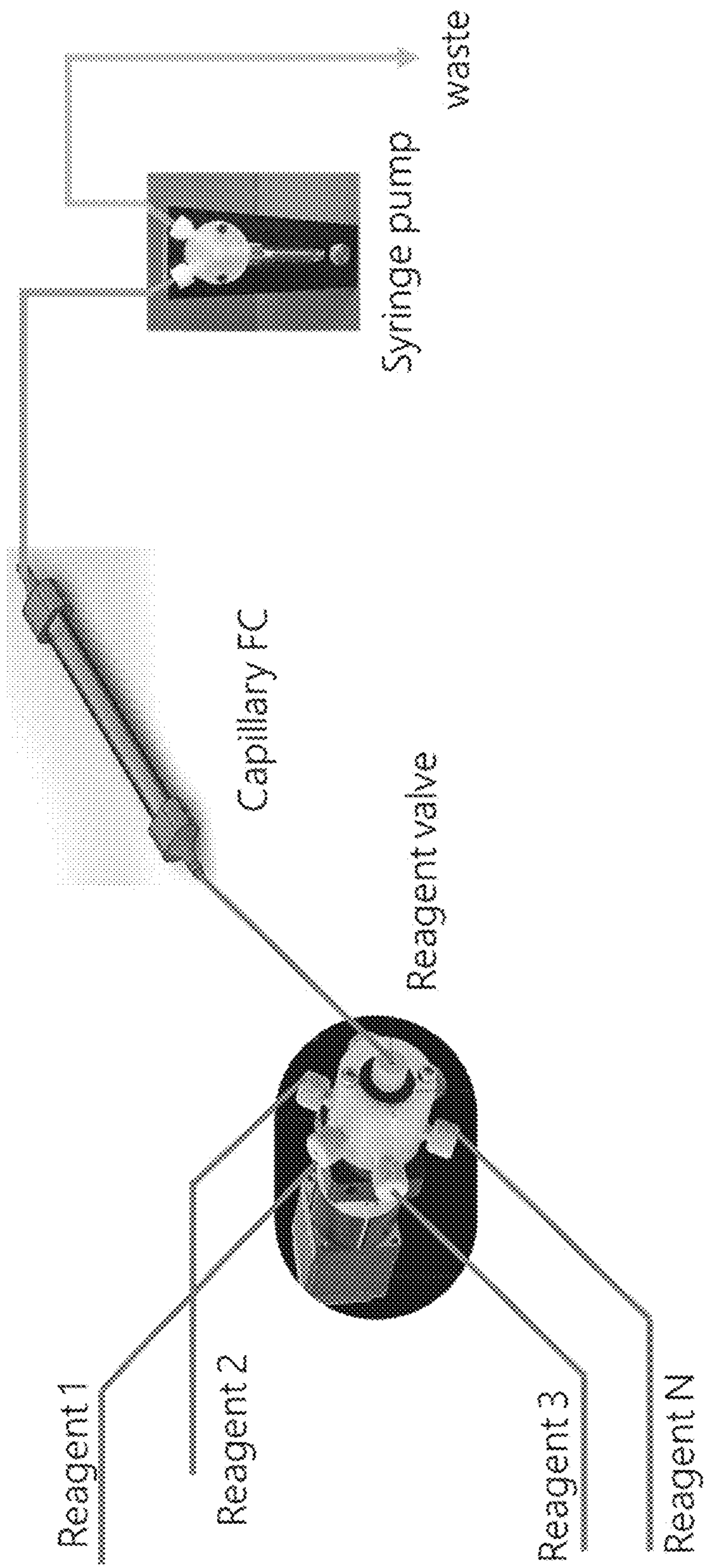


FIG. 31

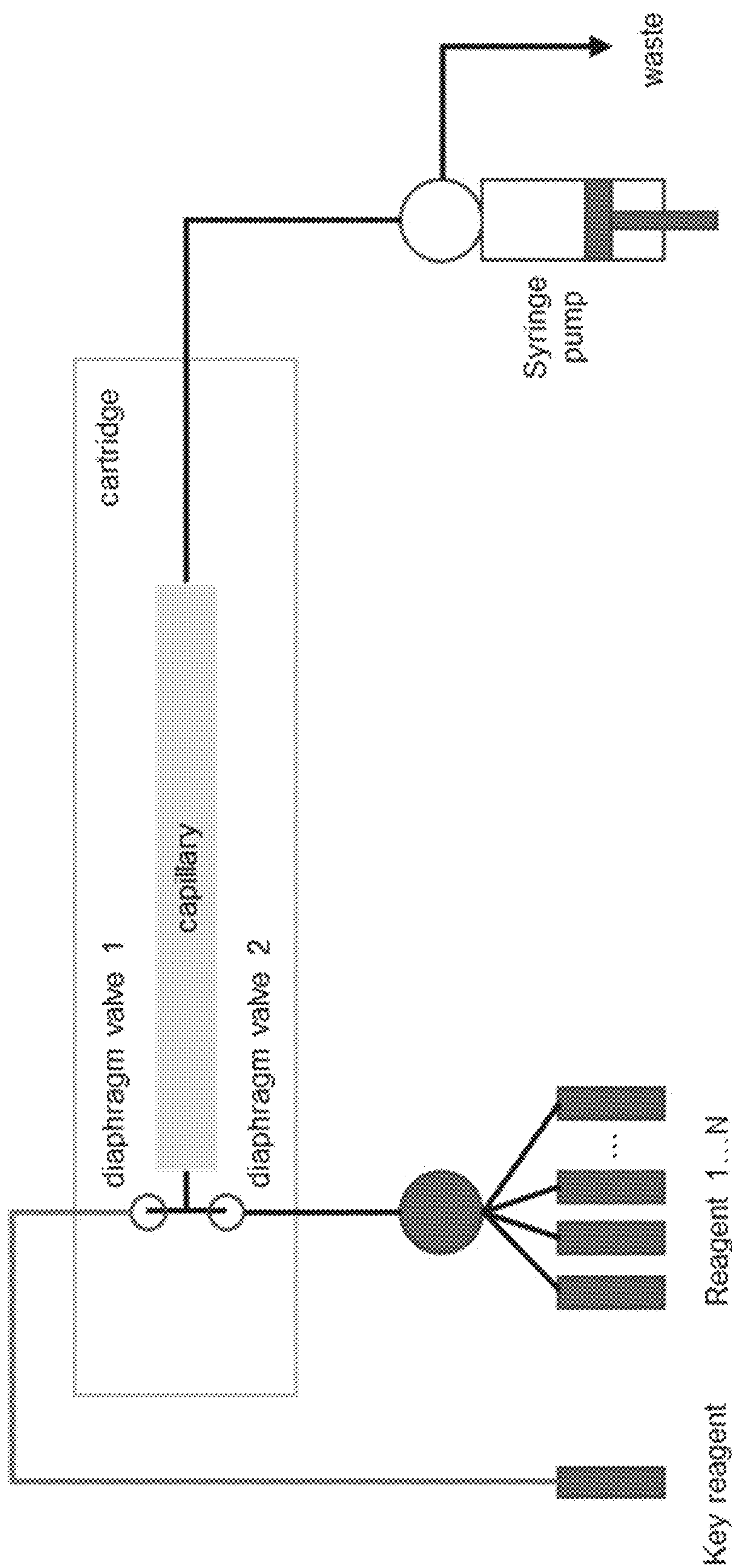


FIG. 32

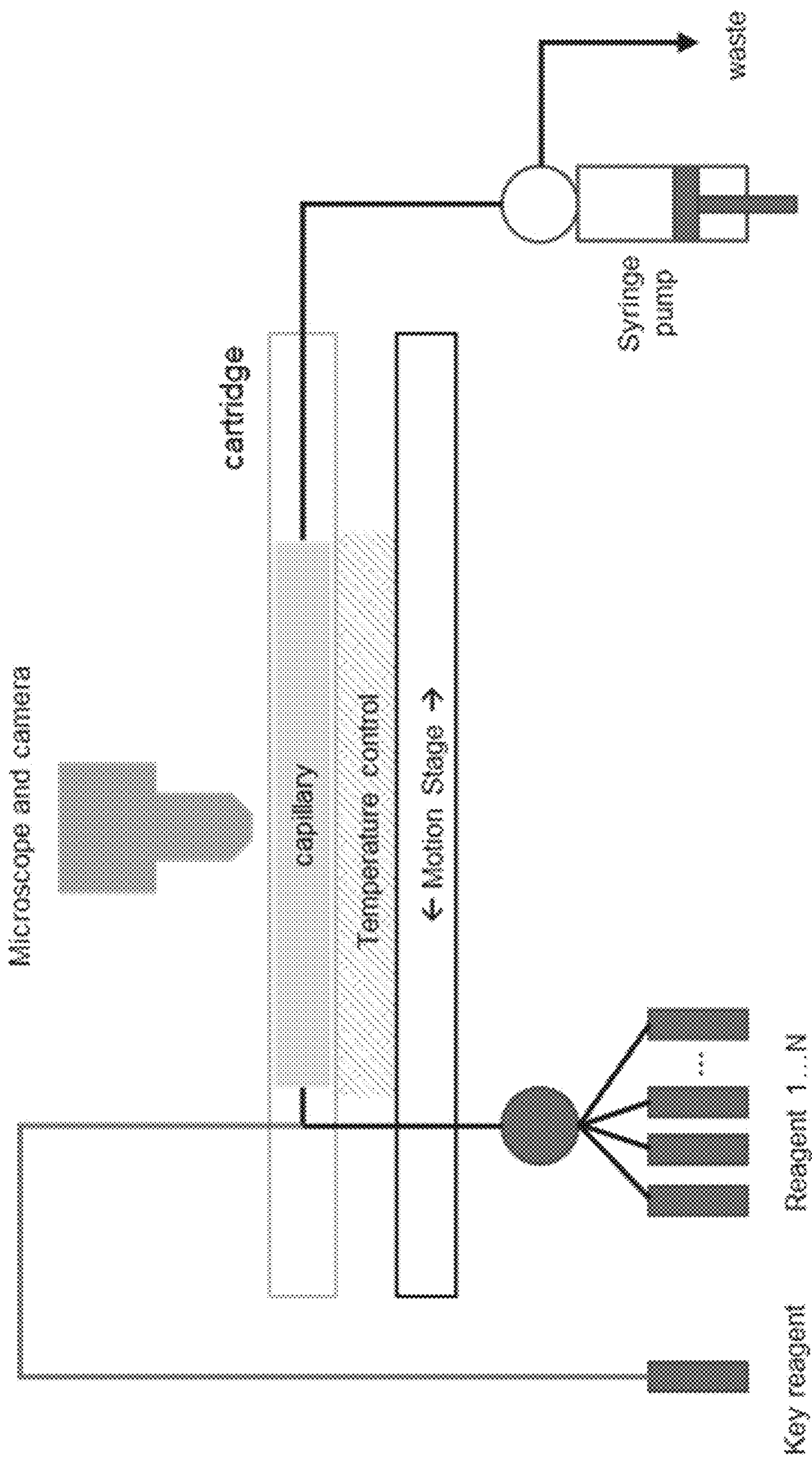


FIG. 33

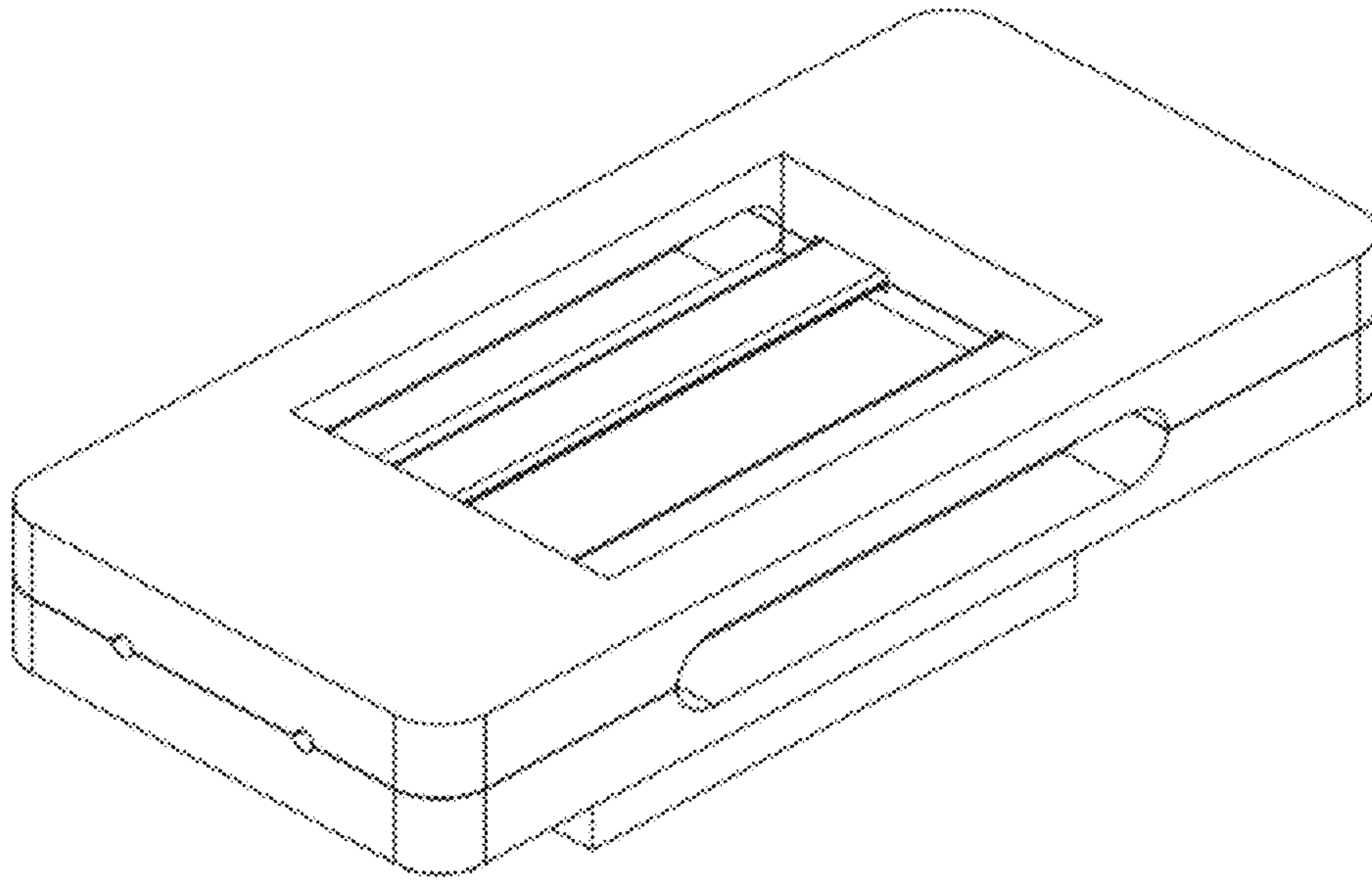


FIG. 34

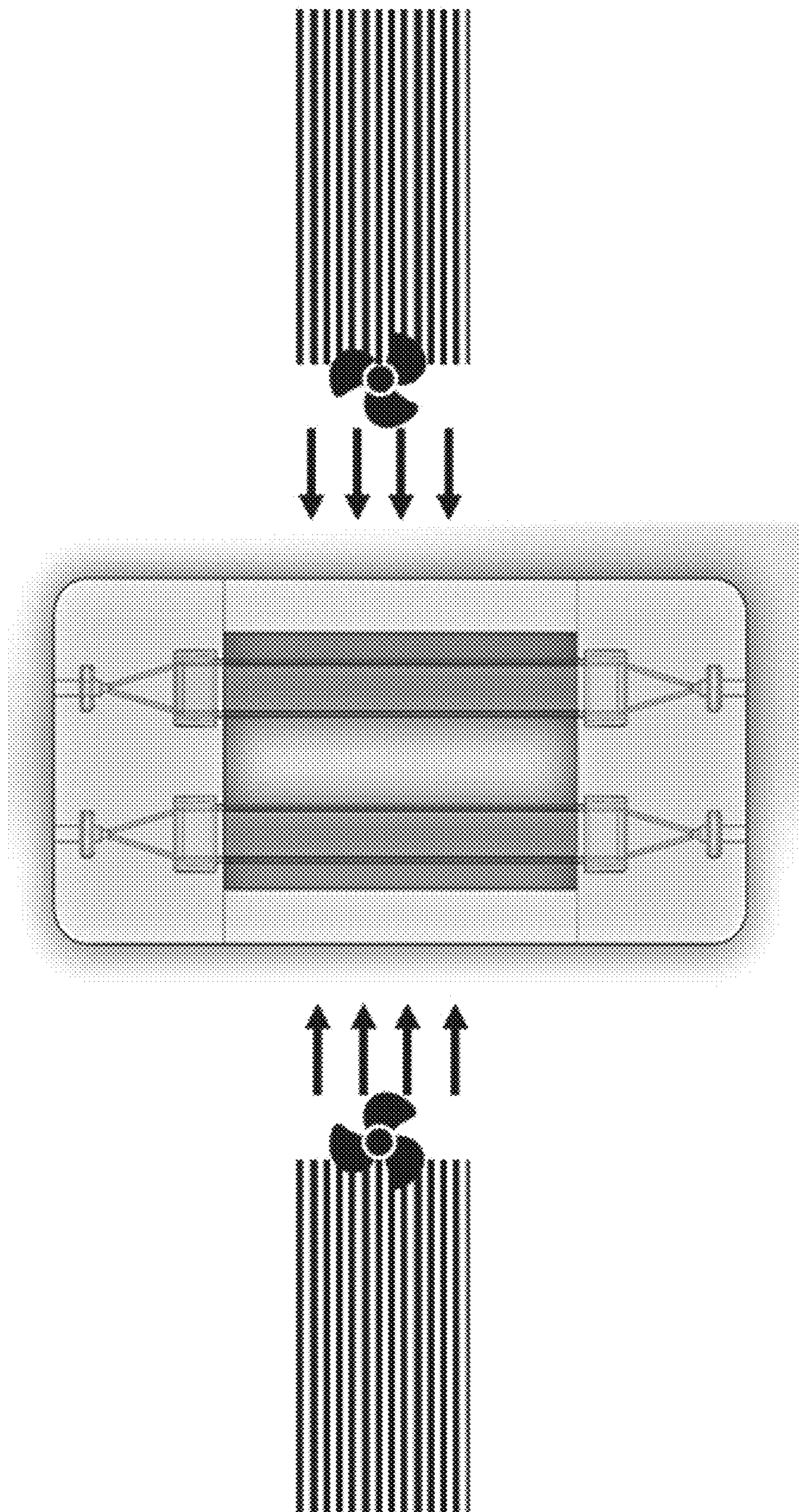


FIG. 35

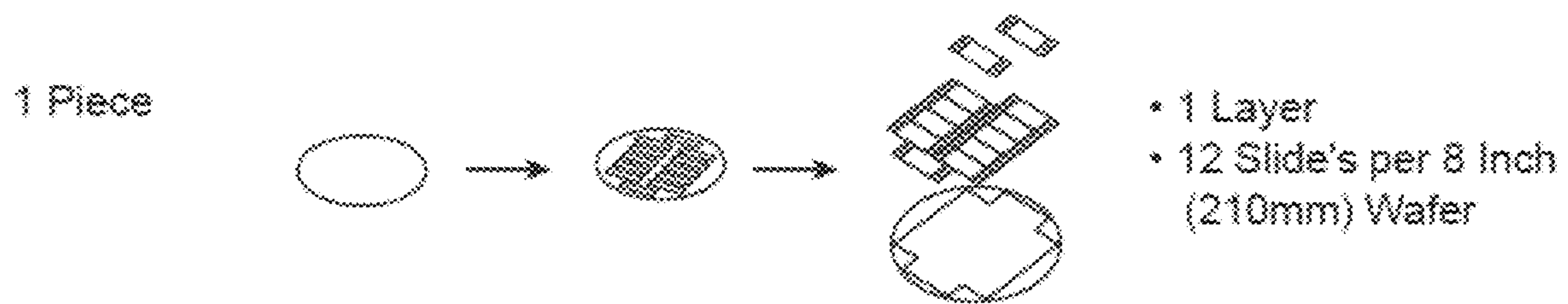


FIG. 36A

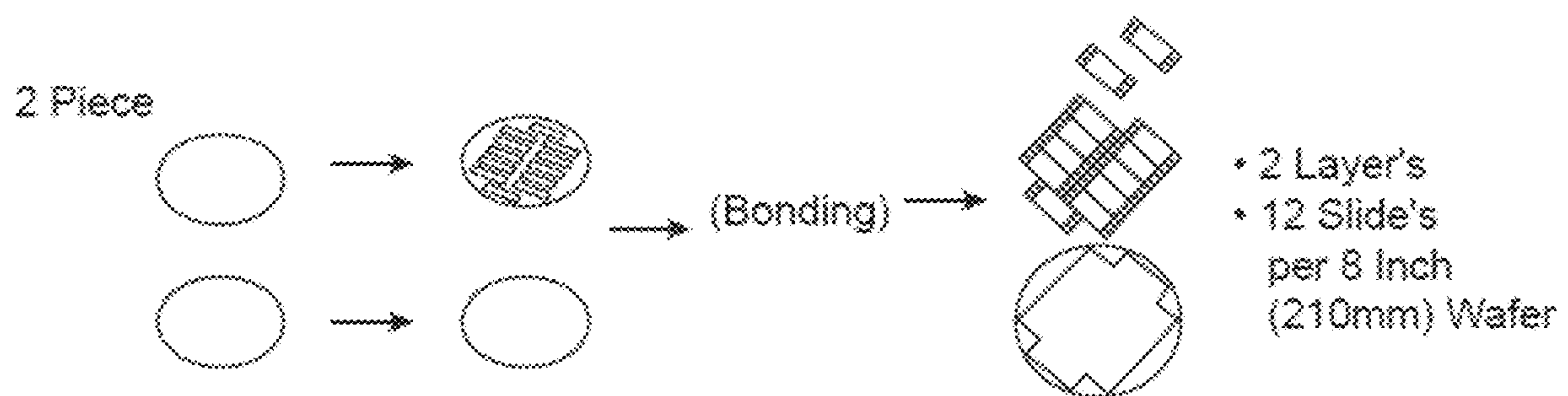


FIG. 36B

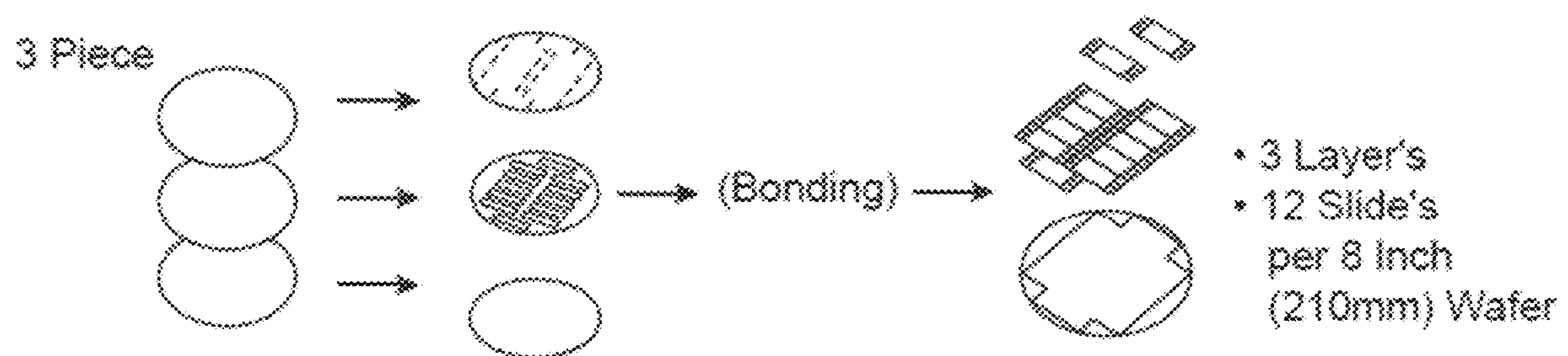


FIG. 36C

Two Lane, One piece FlowCell		
SIZE	Dwg No.	Rev.
B	302_000	@01

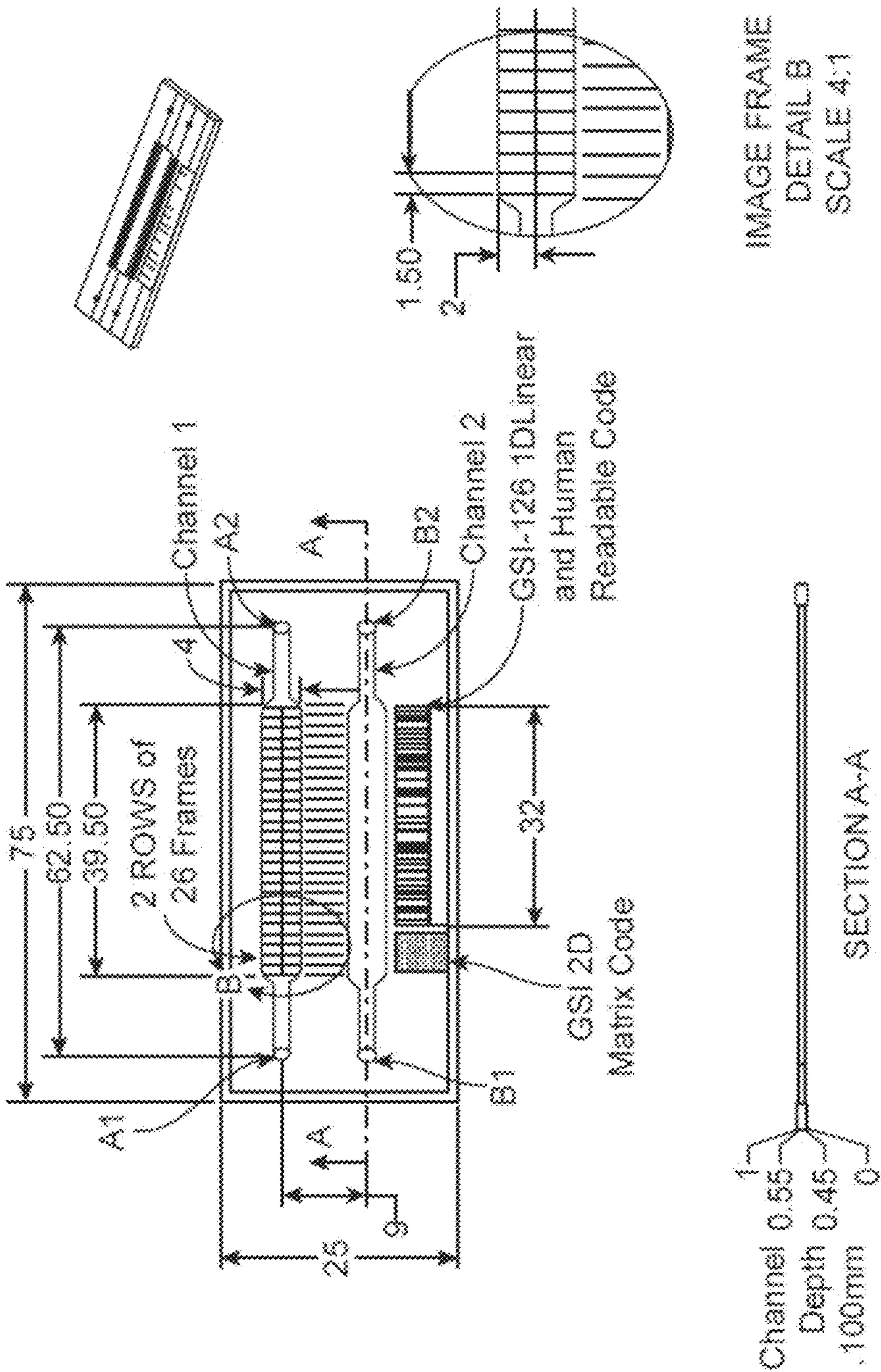
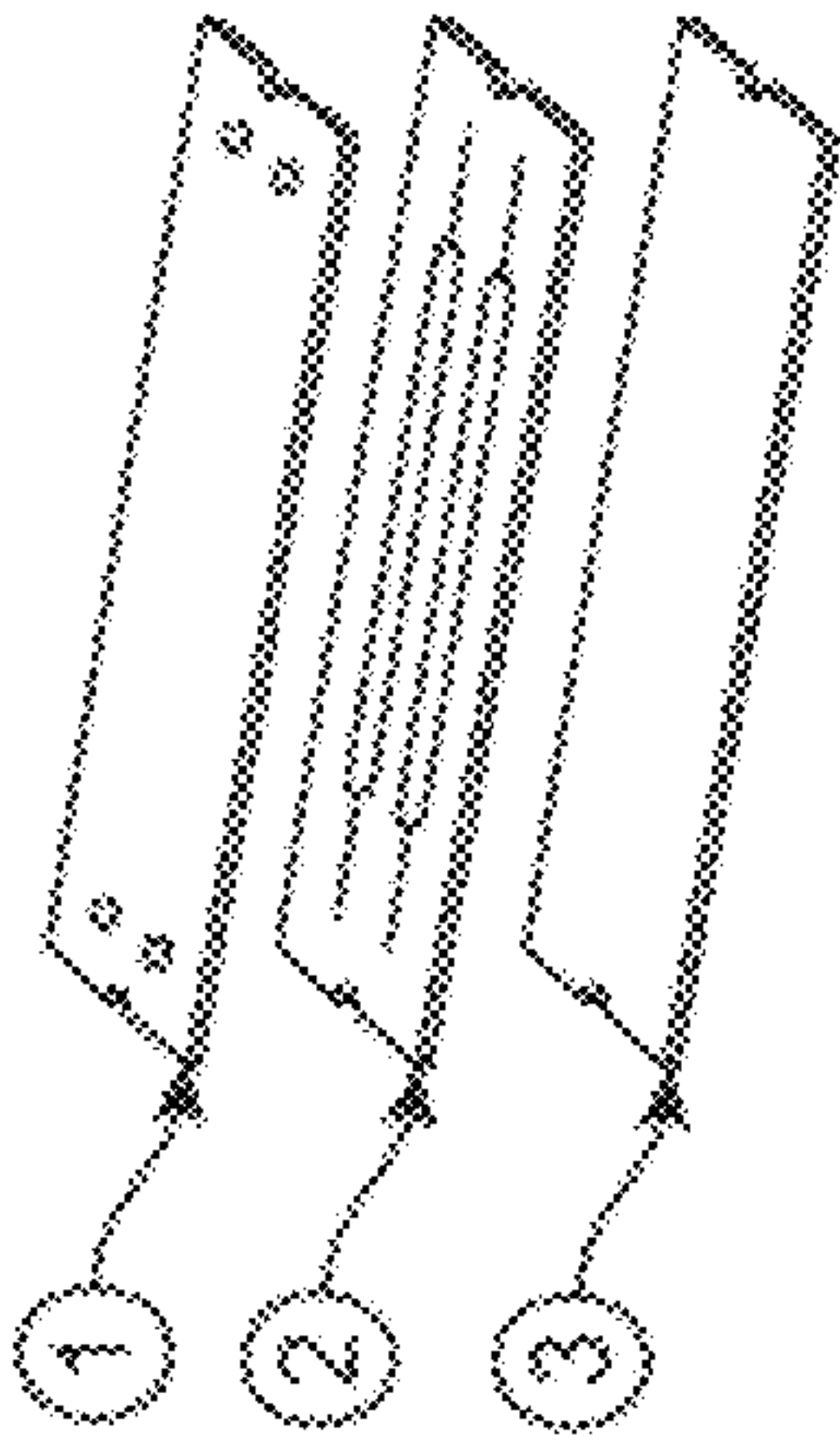
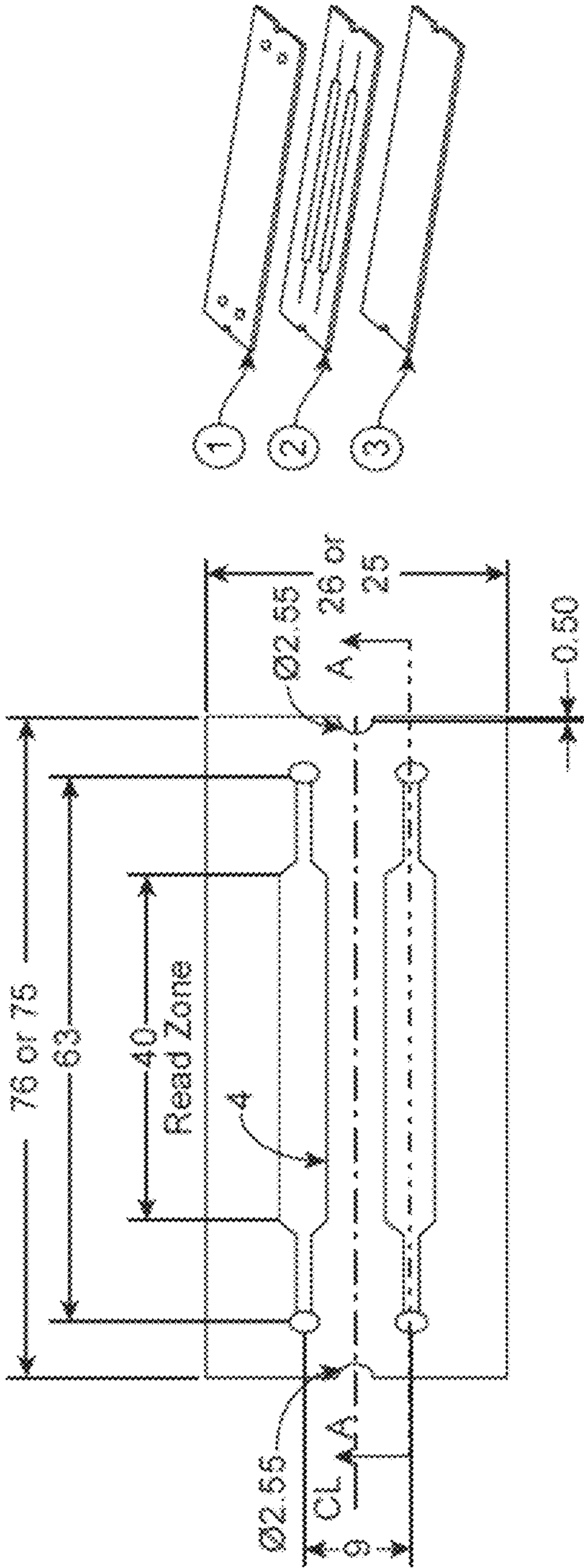


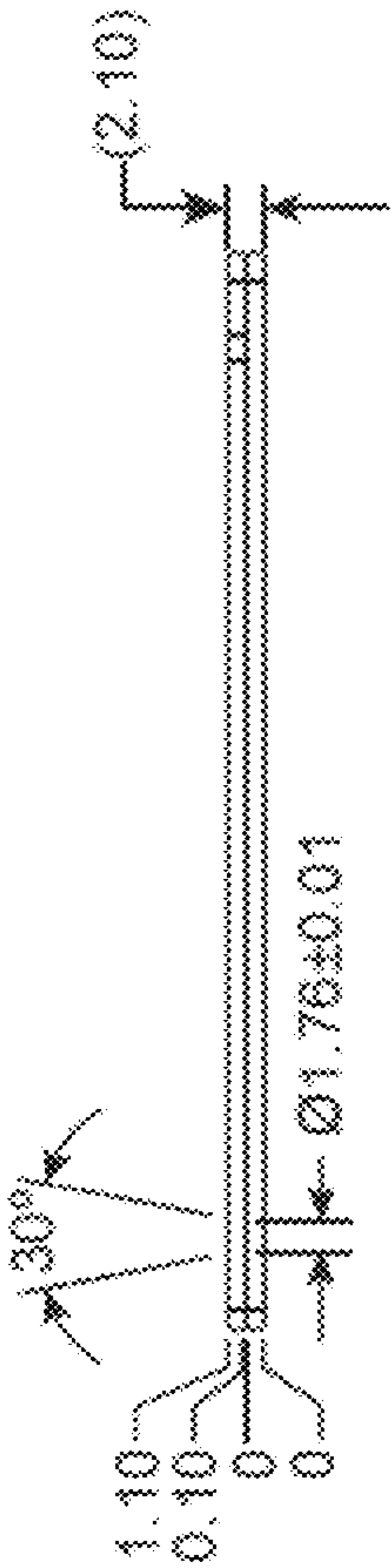
FIG. 37A

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Two Lane, Three piece FlowCell		
SIZE	Dwg No.	Rev.
B	312_000	@01



BOM Table			
ITEM NO.	Number	DESCRIPTION	QTY.
1	311_0001	Inlet Layer, Eight Lane FlowCell	1
2	311_0002	Channel Layer, Eight Lane FlowCell	1
3	311_0003	Base Layer, Eight Lane FlowCell	1



SECTION A-A

FIG. 37C



FIG. 38

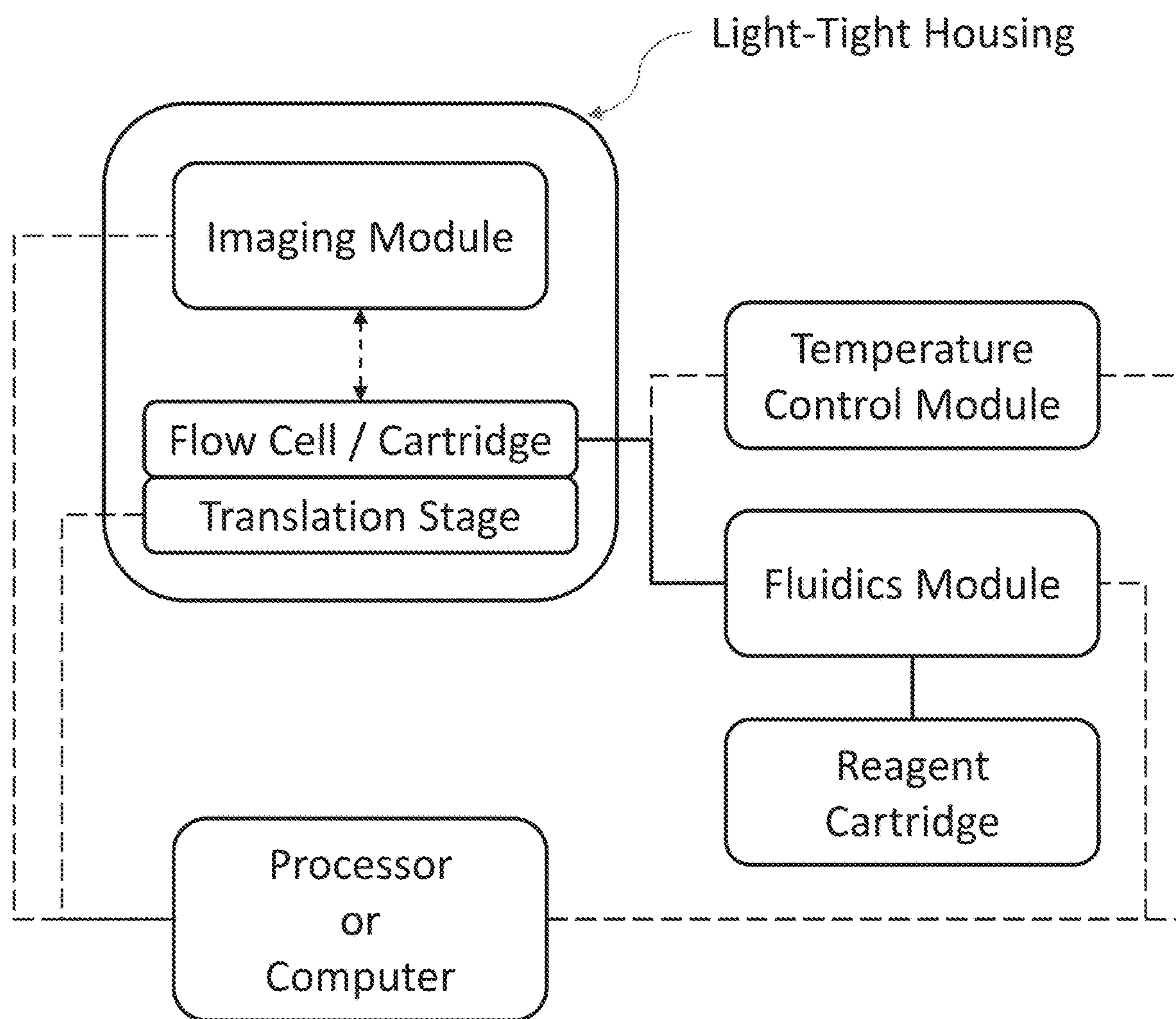


FIG. 39

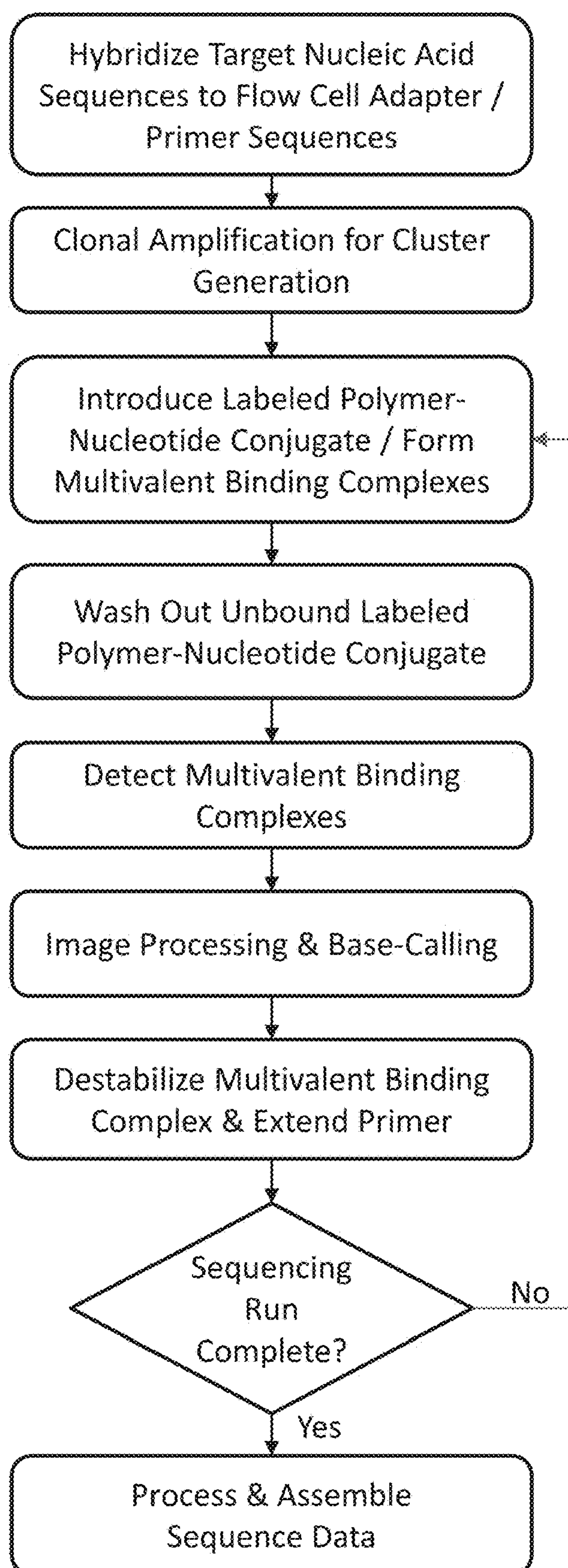


FIG. 40

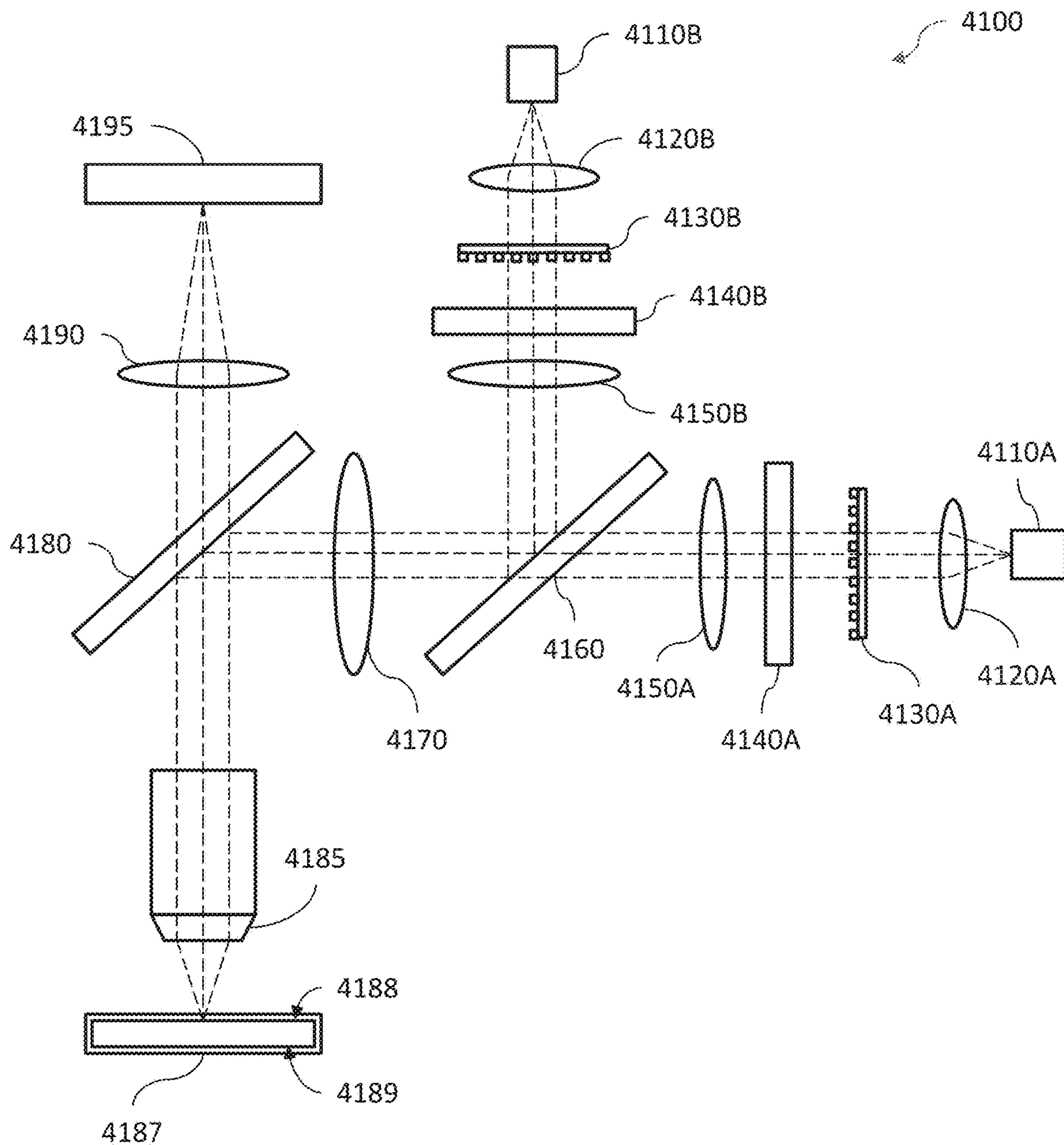


FIG. 41

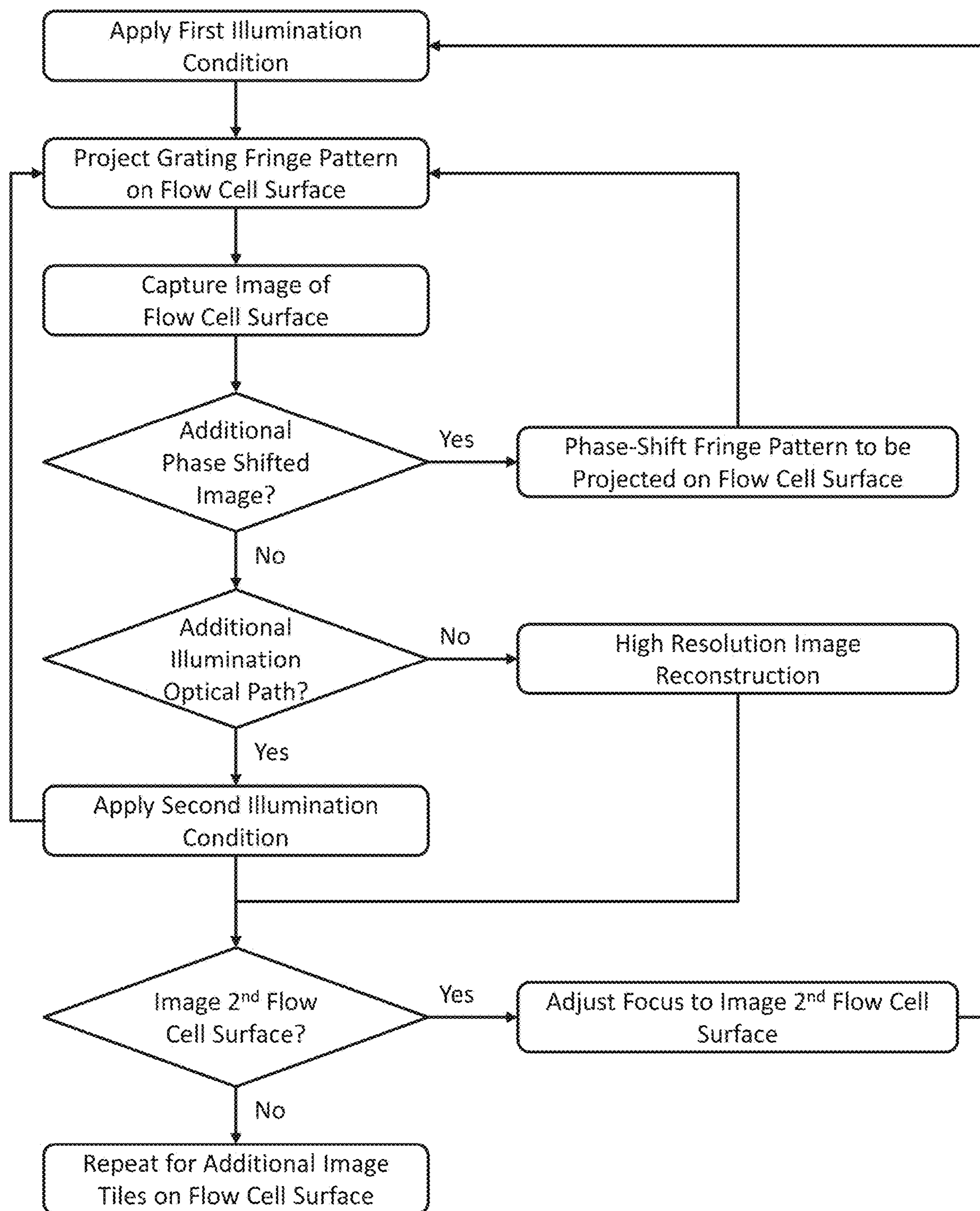


FIG. 42

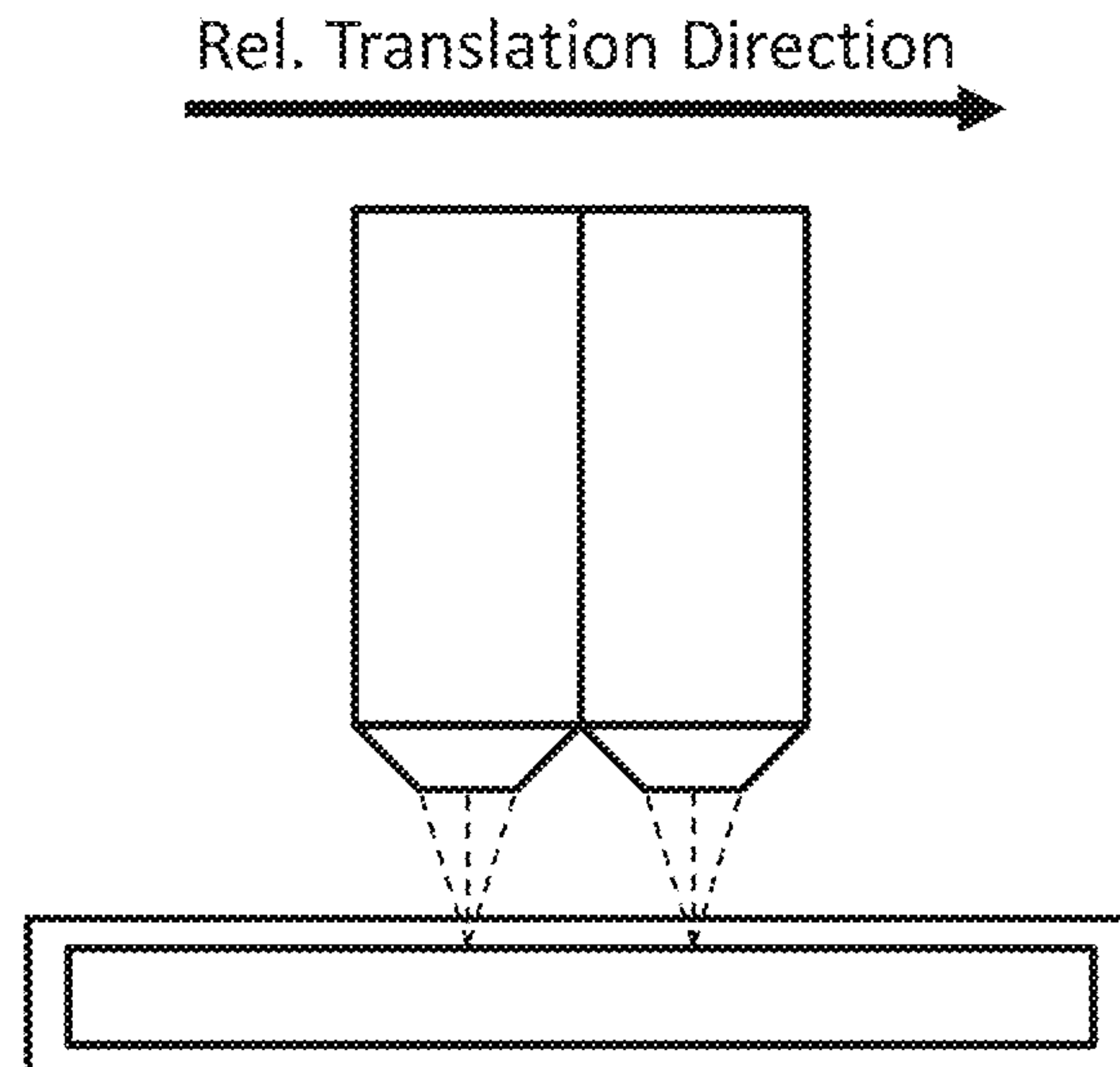


FIG. 43A

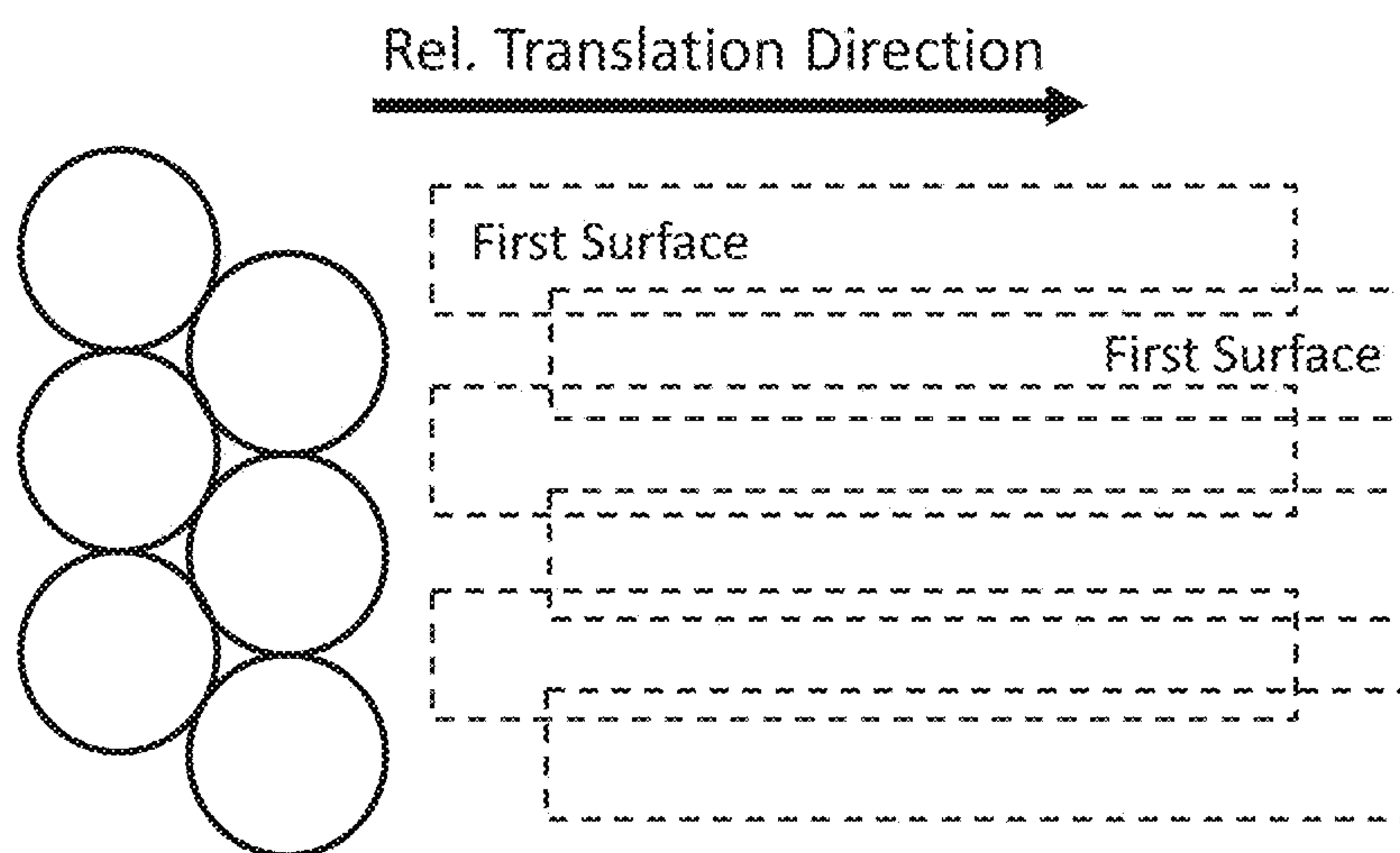


FIG. 43B

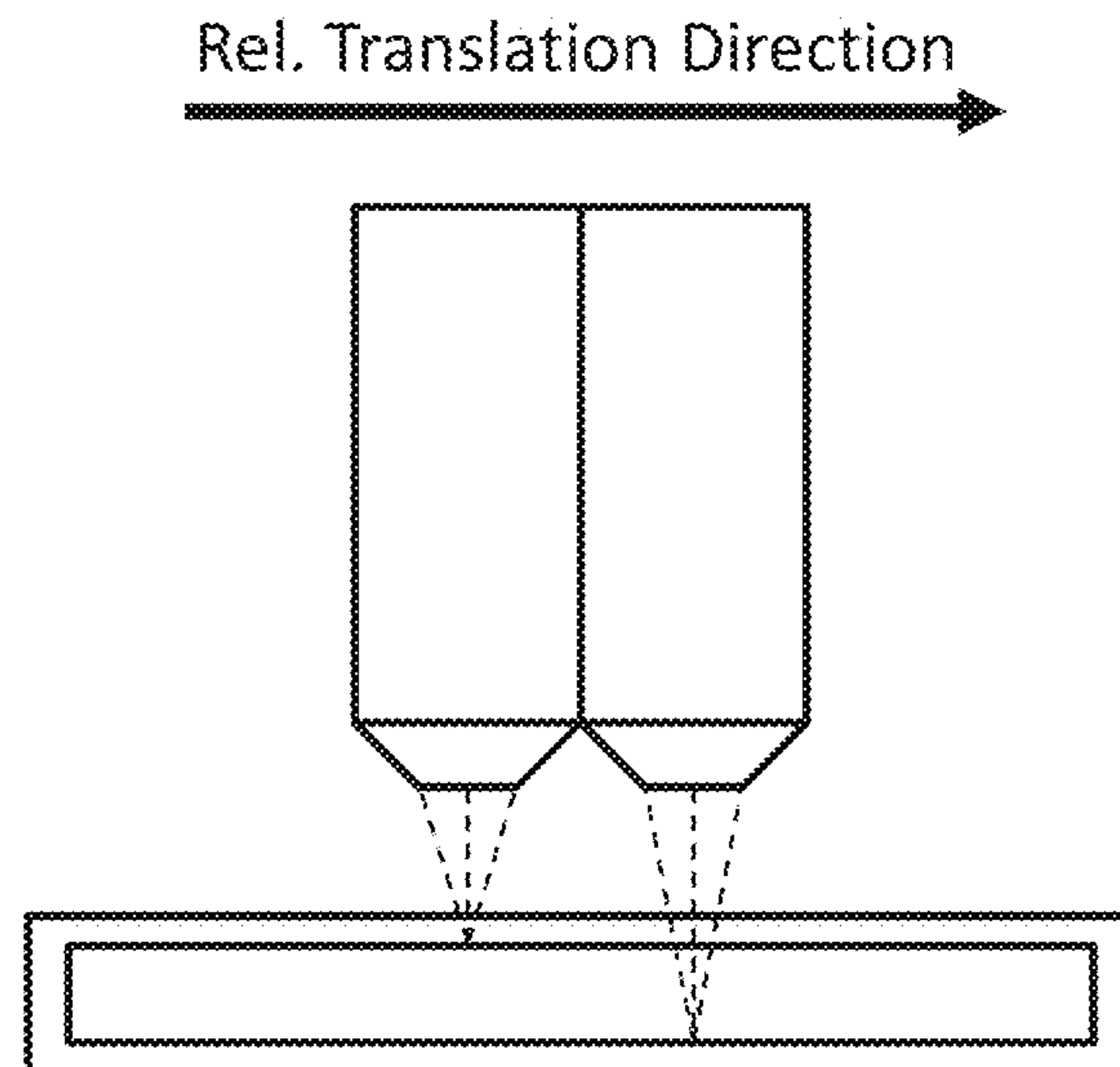


FIG. 44A

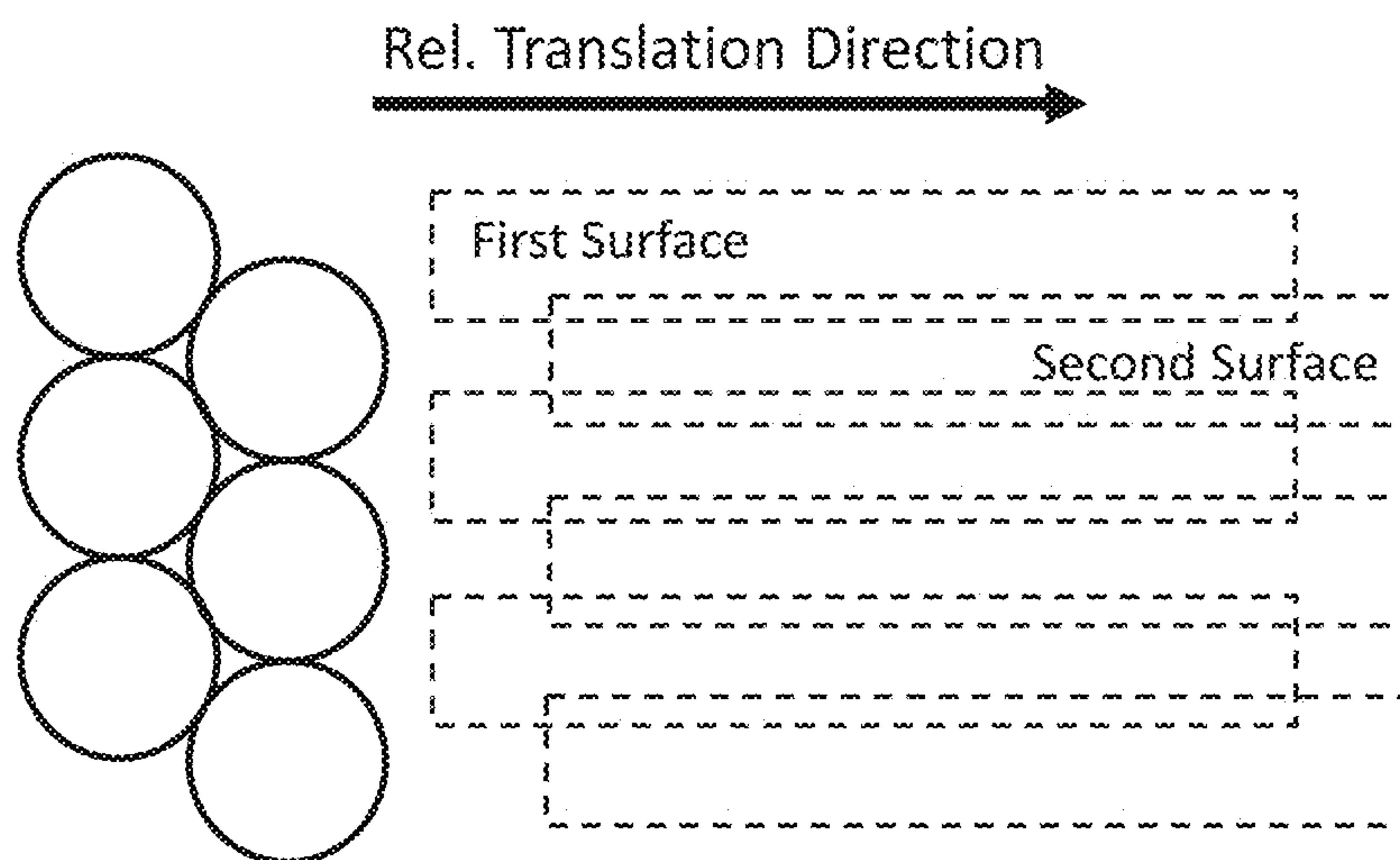


FIG. 44B

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**HIGH PERFORMANCE FLUORESCENCE
IMAGING MODULE FOR GENOMIC
TESTING ASSAY**

CROSS-REFERENCE

This application is a continuation of International Application No. PCT/US2021/013696, filed Jan. 15, 2021, which claims the benefit of U.S. Provisional Application No. 63/076,361, filed Sep. 9, 2020, and U.S. Provisional Application No. 62/962,723, filed Jan. 17, 2020, each of which is incorporated herein by reference in its entirety.

BACKGROUND

In typical fluorescence-based genomic testing assays, e.g., genotyping or nucleic acid sequencing (using either real time, cyclic, or stepwise reaction schemes), dye molecules that are attached to nucleic acid molecules tethered on a substrate are excited using an excitation light source, a fluorescence photon signal is generated in one or more spatially-localized positions on the substrate, and the fluorescence is subsequently imaged through an optical system onto an image sensor. An analysis process is then used to analyze the images, find the positions of labeled molecules (or clonally amplified clusters of molecules) on the substrate, and quantify the fluorescence photon signal in terms of wavelength and spatial coordinates, which may then be correlated with the degree to which a specific chemical reaction, e.g., a hybridization event or base addition event, occurred in the specified locations on the substrate. Imaging-based methods provide large scale parallelism and multiplexing capabilities, which help to drive down the cost and accessibility of such technologies. However, detection errors that arise from, for example, overly dense packing of labeled molecules (or clonally-amplified clusters of molecules) within a small region of the substrate surface, or due to low contrast-to-noise ratio (CNR) in the image, may lead to errors in attributing the fluorescence signal to the correct molecules (or clonally amplified clusters of molecules).

SUMMARY

Disclosed herein are imaging systems configured to image a first interior surface and a second interior surface of a flow cell, the imaging systems comprising: a) an objective lens; b) at least one image sensor; and c) at least one tube lens disposed in an optical path between the objective lens and the at least one image sensor; wherein said optical system has a numerical aperture (NA) of less than 0.6 and a field-of-view (FOV) of greater than 1.0 mm²; and wherein the at least one tube lens is configured to correct imaging performance such that images of the first interior surface of the flow cell and the second interior surface of the flow cell have substantially the same optical resolution.

In some embodiments, the flow cell has a wall thickness of at least 700 μm and a fluid-filled gap between the first interior surface and the second interior surface of at least 50 μm. In some embodiments, the images of the first interior surface and the second interior surface are acquired without moving an optical compensator into an optical path between said objective lens and said at least one image sensor. In some embodiments, the imaging system has a numerical aperture (NA) of less than 0.6. In some embodiments, the imaging system has a numerical aperture (NA) of greater than 0.3. In some embodiments, the imaging system has a field-of-view (FOV) of greater than 1.5 mm². In some

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embodiments, the optical resolution of images of the first interior surface and the second interior surface are diffraction-limited across the entire field-of-view (FOV). In some embodiments, the at least one tube lens comprises, in order, an asymmetric convex-convex lens, a convex-plano lens, an asymmetric concave-concave lens, and an asymmetric convex-concave lens. In some embodiments, the imaging system comprises two or more tube lenses which are designed to provide optimal imaging performance for the first interior surface and the second interior surface at two or more fluorescence wavelengths. In some embodiments, the imaging system further comprises a focusing mechanism configured to refocus the optical system between acquiring images of the first interior surface and the second interior surface. In some embodiments, the imaging system is configured to image two or more fields-of-view on at least one of the first interior surface or the second interior surface. In some embodiments, the first interior surface and second interior surface of the flow cell are coated with a hydrophilic coating layer, and wherein said hydrophilic coating layer further comprises labeled nucleic acid colonies disposed thereon at a surface density of >10,000 nucleic acid colonies/mm². In some embodiments, an image of the first interior surface or second interior surface acquired using the imaging system shows a contrast to noise ratio (CNR) of at least 5 when the nucleic acid colonies are labeled with cyanine dye 3 (Cy3), the imaging system comprises a dichroic mirror and band-pass filter set optimized for Cy3 emission, and the image is acquired under non-signal saturating conditions while the surface is immersed in 25 mM ACES, pH 7.4 buffer. In some embodiments, said imaging system comprises 1, 2, 3, or 4 imaging channels configured to detect nucleic acid colonies disposed on at least one of said two distinct surfaces that have been labeled with 1, 2, 3, or 4 distinct detectable labels. In some embodiments, the imaging system is used to monitor a sequencing-by-avidity, sequencing-by-nucleotide base-pairing, sequencing-by-nucleotide binding, or sequencing-by-nucleotide incorporation reaction on at least one of the first interior surface and the second interior surface and detect a bound or incorporated nucleotide base. In some embodiments, the imaging system is used to perform nucleic acid sequencing. In some embodiments, the imaging system is used to determine a genotype of a sample, wherein determining the genotype of the sample comprises preparing a nucleic acid molecule extracted from the sample for sequencing, and then sequencing the nucleic acid molecule. In some embodiments, the at least one image sensor comprises pixels having a pixel dimension chosen such that a spatial sampling frequency for the imaging system is at least twice an optical resolution of the imaging system. In some embodiments, a combination of the objective lens and the at least one tube lens is configured to optimize a modulation transfer function in the spatial frequency range from 700 cycles per mm to 1100 cycles per mm in the sample plane. In some embodiments, the at least one tube lens is designed to correct modulation transfer function (MTF) at one or more specified spatial frequencies, defocus, spherical aberration, chromatic aberration, coma, astigmatism, field curvature, image distortion, image contrast-to-noise ratio (CNR), or any combination thereof, for a combination of the objective lens and the at least one tube lens.

Also disclosed herein are methods of sequencing a nucleic acid molecule, the methods comprising: a) imaging a first surface and an axially-displaced second surface using an optical system which comprises an objective lens and at least one image sensor, wherein said optical system has a numerical aperture (NA) of less than 0.6 and a field-of-view

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(FOV) of greater than 1.0 mm^2 , and wherein images of the first surface and the axially-displaced second surface having substantially the same optical resolution are acquired without moving an optical compensator into an optical path between said objective lens and said at least one image sensor; and b) detecting a fluorescently-labeled composition comprising the nucleic acid molecule, or a complement thereof, disposed on the first surface or the axially-displaced second surface to determine an identity of a nucleotide in the nucleic acid molecule.

In some embodiments, a focusing mechanism is utilized to refocus the optical system between acquiring images of the first surface and the axially-displaced second surface. In some embodiments, the method further comprises imaging two or more fields-of-view on at least one of the first surface or axially-displaced second surface. In some embodiments, the first surface and the axially-displaced second surface comprise two surfaces of a flow cell. In some embodiments, said two surfaces of the flow cell are coated with a hydrophilic coating layer. In some embodiments, said hydrophilic coating layer further comprises labeled nucleic acid colonies disposed thereon at a surface density of $>10,000$ nucleic acid colonies/ mm^2 . In some embodiments, an image of a surface of said two surfaces acquired using said optical system shows a contrast to noise ratio (CNR) of at least 5 when the nucleic acid colonies are labeled with cyanine dye 3 (Cy3), the optical system comprises a dichroic mirror and bandpass filter set optimized for Cy3 emission, and the image is acquired under non-signal saturating conditions while the surface is immersed in 25 mM ACES, pH 7.4 buffer. In some embodiments, said optical system comprises 1, 2, 3, or 4 imaging channels configured to detect nucleic acid colonies disposed on at least one of the first surface and the axially-displaced second surface that have been labeled with 1, 2, 3, or 4 distinct detectable labels. In some embodiments, the at least one image sensor comprises pixels having a pixel dimension chosen such that a spatial sampling frequency for the optical system is at least twice an optical resolution of the optical system. In some embodiments, the optical system comprises at least one tube lens positioned between the objective lens and the at least one image sensor, and wherein the at least one tube lens is configured to correct an imaging performance metric for imaging a first interior surface of a flow cell and a second interior surface of the flow cell. In some embodiments, the flow cell has a wall thickness of at least $700 \text{ }\mu\text{m}$ and a gap between the first interior surface and the second interior surface of at least $50 \text{ }\mu\text{m}$. In some embodiments, the at least one tube lens comprises, in order, an asymmetric convex-convex lens, a convex-plano lens, an asymmetric concave-concave lens, and an asymmetric convex-concave lens. In some embodiments, the optical system comprises two or more tube lenses which are designed to provide optimal imaging performance at two or more fluorescence wavelengths. In some embodiments, a combination of objective lens and tube lens is configured to optimize a modulation transfer function in the mid to high spatial frequency range. In some embodiments, the imaging performance metric comprises a measurement of modulation transfer function (MTF) at one or more specified spatial frequencies, defocus, spherical aberration, chromatic aberration, coma, astigmatism, field curvature, image distortion, image contrast-to-noise ratio (CNR), or any combination thereof. In some embodiments, the optical resolution of images of the first surface and axially-displaced second surface are diffraction-limited across the entire field-of-view (FOV). In some embodiments, the sequencing of the nucleic acid molecule further comprises performing a sequencing-

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by-avidity, sequencing-by-nucleotide base-pairing, sequencing-by-nucleotide binding, or sequencing-by-nucleotide incorporation reaction on at least one of the first surface and axially-displaced second surface and detecting a bound or incorporated nucleotide base. In some embodiments, the method further comprises determining a genotype of a sample, wherein determining the genotype of the sample comprises preparing said nucleic acid molecule for sequencing, and then sequencing said nucleic acid molecule.

Disclosed herein are imaging systems configured to image two distinct, axially-displaced surfaces, the imaging systems comprising an objective lens and at least one image sensor, wherein said imaging system has a numerical aperture (NA) of less than 0.6 and a field-of-view (FOV) of greater than 1.0 mm^2 , and wherein said imaging system is capable of acquiring images of the two distinct, axially-displaced surfaces that have substantially the same optical resolution without moving an optical compensator into an optical path between said objective lens and said at least one image sensor.

In some embodiments, the imaging system has a numerical aperture of greater than 0.3. In some embodiments, the imaging system further comprises a focusing mechanism used to refocus the optical system between acquiring images of the two distinct, axially-displaced surfaces. In some embodiments, the imaging system is configured to image two or more fields-of-view on at least one of said two distinct, axially-displaced surfaces. In some embodiments, said two distinct, axially-displaced surfaces comprise two surfaces of a flow cell. In some embodiments, said two distinct surfaces of the flow cell are coated with a hydrophilic coating layer, and wherein said hydrophilic coating layer further comprises labeled nucleic acid colonies disposed thereon at a surface density of $>10,000$ nucleic acid colonies/ mm^2 . In some embodiments, said imaging system comprises 1, 2, 3, or 4 imaging channels configured to detect nucleic acid colonies disposed on at least one of said two distinct surfaces that have been labeled with 1, 2, 3, or 4 distinct detectable labels. In some embodiments, the at least one image sensor comprises pixels having a pixel dimension chosen such that a spatial sampling frequency for the imaging system is at least twice an optical resolution of the imaging system. In some embodiments, the imaging system comprises at least one tube lens positioned between the objective lens and the at least one image sensor, and wherein the at least one tube lens is configured to correct an imaging performance metric for imaging a first interior surface of a flow cell and a second interior surface of the flow cell. In some embodiments, the flow cell has a wall thickness of at least $700 \text{ }\mu\text{m}$ and a gap between the first interior surface and the second interior surface of at least $50 \text{ }\mu\text{m}$. In some embodiments, the imaging system comprises two or more tube lenses which are designed to provide optimal imaging performance at two or more fluorescence wavelengths. In some embodiments, the optical resolution of images of the two distinct, axially-displaced surfaces are diffraction-limited across the entire field-of-view (FOV).

Disclosed herein are methods of sequencing a nucleic acid molecule, the method comprising: a) imaging a first surface and an axially-displaced second surface using a compensation-free optical system which comprises an objective lens and at least one image sensor, wherein said optical system has a numerical aperture (NA) of less than 0.6 and a field-of-view (FOV) of greater than 1.0 mm^2 ; b) processing the images of the first surface and the axially-displaced second surface to correct for optical aberration such that the images of the first surface and the axially-displaced second surface have substantially the same optical resolution; and c)

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detecting a fluorescently-labeled composition comprising the nucleic acid molecule, or a complement thereof, disposed on the first surface or the axially-displaced second surface to determine an identity of a nucleotide in the nucleic acid molecule.

In some embodiments, the images of the first surface and the axially-displaced second surface are acquired without moving an optical compensator into an optical path between said objective lens and said at least one image sensor. In some embodiments, the images of the first surface and the axially-displaced second surface are acquired by just re-focusing the optical system. In some embodiments, the method further comprises imaging two or more fields-of-view on at least one of the first surface or axially-displaced second surface. In some embodiments, the first surface and the axially-displaced second surface comprise two surfaces of a flow cell. In some embodiments, said two surfaces of the flow cell are coated with a hydrophilic coating layer. In some embodiments, said hydrophilic coating layer further comprises labeled nucleic acid colonies disposed thereon at a surface density of $>10,000$ nucleic acid colonies/mm². In some embodiments, an image of a surface of said two surfaces acquired using said optical system shows a contrast to noise ratio (CNR) of at least 5 when the nucleic acid colonies are labeled with cyanine dye 3 (Cy3), the optical system comprises a dichroic mirror and bandpass filter set optimized for Cy3 emission, and the image is acquired under non-signal saturating conditions while the surface is immersed in 25 mM ACES, pH 7.4 buffer. In some embodiments, said optical system comprises 1, 2, 3, or 4 imaging channels configured to detect nucleic acid colonies disposed on at least one of the first surface and the axially-displaced second surface that have been labeled with 1, 2, 3, or 4 distinct detectable labels. In some embodiments, at least one image sensor comprises pixels having a pixel dimension chosen such that a spatial sampling frequency for the optical system is at least twice an optical resolution of the optical system. In some embodiments, the optical system comprises at least one tube lens positioned between the objective lens and the at least one image sensor, and wherein the at least one tube lens is configured to correct an imaging performance metric for imaging a first interior surface of a flow cell and a second interior surface of the flow cell. In some embodiments, the flow cell has a wall thickness of at least 700 μm and a gap between the first interior surface and the second interior surface of at least 50 μm . In some embodiments, the at least one tube lens comprises, in order, an asymmetric convex-convex lens, a convex-plano lens, an asymmetric concave-concave lens, and an asymmetric convex-concave lens. In some embodiments, the optical system comprises two or more tube lenses which are designed to provide optimal imaging performance at two or more fluorescence wavelengths. In some embodiments, a combination of objective lens and tube lens is configured to optimize a modulation transfer function in the mid to high spatial frequency range. In some embodiments, the imaging performance metric comprises a measurement of modulation transfer function (MTF) at one or more specified spatial frequencies, defocus, spherical aberration, chromatic aberration, coma, astigmatism, field curvature, image distortion, image contrast-to-noise ratio (CNR), or any combination thereof. In some embodiments, the optical resolution of images of the first surface and axially-displaced second surface are diffraction-limited across the entire field-of-view (FOV). In some embodiments, the sequencing of the nucleic acid molecule further comprises performing a sequencing-

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ing-by-nucleotide incorporation reaction on at least one of the first surface and axially-displaced second surface and detecting a bound or incorporated nucleotide base. In some embodiments, the method further comprises determining a genotype of a sample, wherein determining the genotype of the sample comprises preparing said nucleic acid molecule for sequencing, and then sequencing said nucleic acid molecule.

Disclosed herein are systems for sequencing a nucleic acid molecule comprising: a) an optical system comprising an objective lens and at least one image sensor, wherein said optical system has a numerical aperture (NA) of less than 0.6 and a field-of-view (FOV) of greater than 1.0 mm², and is configured to acquire images of a first surface and an axially-displaced second surface; and b) a processor programmed to: i) process images of the first surface and the axially-displaced second surface to correct for optical aberration such that the images of the first surface and the axially-displaced second surface have substantially the same optical resolution; and ii) detect a fluorescently-labeled composition comprising the nucleic acid molecule, or a complement thereof, disposed on the first surface or the axially-displaced second surface to determine an identity of a nucleotide in the nucleic acid molecule.

In some embodiments, the images of the first surface and the axially-displaced second surface are acquired without moving an optical compensator into an optical path between said objective lens and said at least one image sensor. In some embodiments, the images of the first surface and the axially-displaced second surface are acquired by just re-focusing the optical system. In some embodiments, the imaging system has a numerical aperture of greater than 0.3. In some embodiments, the first surface and axially-displaced second surface comprise two surfaces of a flow cell. In some embodiments, said two surfaces of the flow cell are coated with a hydrophilic coating layer, and wherein said hydrophilic coating layer further comprises labeled nucleic acid colonies disposed thereon at a surface density of $>10,000$ nucleic acid colonies/mm². In some embodiments, said optical system comprises 1, 2, 3, or 4 imaging channels configured to detect nucleic acid colonies disposed on at least one of the first surface or axially-displaced second surface that have been labeled with 1, 2, 3, or 4 distinct detectable labels. In some embodiments, the at least one image sensor comprises pixels having a pixel dimension chosen such that a spatial sampling frequency for the optical system is at least twice an optical resolution of the optical system. In some embodiments, the system comprises at least one tube lens positioned between the objective lens and the at least one image sensor, and wherein the at least one tube lens is configured to correct an imaging performance metric for imaging a first interior surface of a flow cell and a second interior surface of the flow cell. In some embodiments, the flow cell has a wall thickness of at least 700 μm and a gap between the first interior surface and the second interior surface of at least 50 μm . In some embodiments, the optical system comprises two or more tube lenses which are designed to provide optimal imaging performance at two or more fluorescence wavelengths.

Disclosed herein are fluorescence imaging systems comprising: a) at least one light source configured to provide excitation light within one or more specified wavelength ranges; b) an objective lens configured to collect fluorescence arising from within a specified field-of-view of a sample plane upon exposure of the sample plane to the excitation light, wherein a numerical aperture of the objective lens is at least 0.3, wherein a working distance of the

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objective lens is at least 700 μm , and wherein the field-of-view has an area of at least 2 mm^2 ; and c) at least one image sensor, wherein the fluorescence collected by the objective lens is imaged onto the image sensor, and wherein a pixel dimension for the image sensor is chosen such that a spatial sampling frequency for the fluorescence imaging system is at least twice an optical resolution of the fluorescence imaging system.

In some embodiments, the numerical aperture is at least 0.75. In some embodiments, the numerical aperture is at least 1.0. In some embodiments, the working distance is at least 850 μm . In some embodiments, the working distance is at least 1,000 μm . In some embodiments, the field-of-view has an area of at least 2.5 mm^2 . In some embodiments, the field-of-view has an area of at least 3 mm^2 . In some embodiments, the spatial sampling frequency is at least 2.5 times the optical resolution of the fluorescence imaging system. In some embodiments, the spatial sampling frequency is at least 3 times the optical resolution of the fluorescence imaging system. In some embodiments, the system further comprises an X-Y-Z translation stage such that the system is configured to acquire a series of two or more fluorescence images in an automated fashion, wherein each image of the series is acquired for a different field-of-view. In some embodiments, a position of the sample plane is simultaneously adjusted in an X direction, a Y direction, and a Z direction to match the position of an objective lens focal plane in between acquiring images for different fields-of-view. In some embodiments, the time required for the simultaneous adjustments in the X direction, Y direction, and Z direction is less than 0.4 seconds. In some embodiments, the system further comprises an autofocus mechanism configured to adjust the focal plane position prior to acquiring an image of a different field-of-view if an error signal indicates that a difference in the position of the focal plane and the sample plane in the Z direction is greater than a specified error threshold. In some embodiments, the specified error threshold is 100 nm. In some embodiments, the specified error threshold is 50 nm. In some embodiments, the system comprises three or more image sensors, and wherein the system is configured to image fluorescence in each of three or more wavelength ranges onto a different image sensor. In some embodiments, a difference in the position of a focal plane for each of the three or more image sensors and the sample plane is less than 100 nm. In some embodiments, a difference in the position of a focal plane for each of the three or more image sensors and the sample plane is less than 50 nm. In some embodiments, the total time required to reposition the sample plane, adjust focus if necessary, and acquire an image is less than 0.4 seconds per field-of-view. In some embodiments, the total time required to reposition the sample plane, adjust focus if necessary, and acquire an image is less than 0.3 seconds per field-of-view.

Also disclosed herein are fluorescence imaging systems for dual-side imaging of a flow cell comprising: a) an objective lens configured to collect fluorescence arising from within a specified field-of-view of a sample plane within the flow cell; b) at least one tube lens positioned between the objective lens and at least one image sensor, wherein the at least one tube lens is configured to correct an imaging performance metric for a combination of the objective lens, the at least one tube lens, and the at least one image sensor when imaging an interior surface of the flow cell, and wherein the flow cell has a wall thickness of at least 700 μm and a gap between an upper interior surface and a lower interior surface of at least 50 μm ; wherein the imaging performance metric is substantially the same for imaging the

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upper interior surface or the lower interior surface of the flow cell without moving an optical compensator into or out of an optical path between the flow cell and the at least one image sensor, without moving one or more optical elements of the tube lens along the optical path, and without moving one or more optical elements of the tube lens into or out of the optical path.

In some embodiments, the objective lens is a commercially-available microscope objective. In some embodiments, the commercially-available microscope objective has a numerical aperture of at least 0.3. In some embodiments, the objective lens has a working distance of at least 700 μm . In some embodiments, the objective lens is corrected to compensate for a cover slip thickness (or flow cell wall thickness) of 0.17 mm. In some embodiments, the fluorescence imaging system further comprising an electro-optical phase plate positioned adjacent to the objective lens and between the objective lens and the tube lens, wherein the electro-optical phase plate provides correction for optical aberrations caused by a fluid filling the gap between the upper interior surface and the lower interior surface of the flow cell. In some embodiments, the at least one tube lens is a compound lens comprising three or more optical components. In some embodiments, the at least one tube lens is a compound lens comprising four optical components. In some embodiments, the four optical components comprise, in order, a first asymmetric convex-convex lens, a second convex-plano lens, a third asymmetric concave-concave lens, and a fourth asymmetric convex-concave lens. In some embodiments, the at least one tube lens is configured to correct an imaging performance metric for a combination of the objective lens, the at least one tube lens, and the at least one image sensor when imaging an interior surface of a flow cell having a wall thickness of at least 1 mm. In some embodiments, the at least one tube lens is configured to correct an imaging performance metric for a combination of the objective lens, the at least one tube lens, and the at least one image sensor when imaging an interior surface of a flow cell having a gap of at least 100 μm . In some embodiments, the at least one tube lens is configured to correct an imaging performance metric for a combination of the objective lens, the at least one tube lens, and the at least one image sensor when imaging an interior surface of a flow cell having a gap of at least 200 μm . In some embodiments, the system comprises a single objective lens, two tube lenses, and two image sensors, and each of the two tube lenses is designed to provide optimal imaging performance at a different fluorescence wavelength. In some embodiments, the system comprises a single objective lens, three tube lenses, and three image sensors, and each of the three tube lenses is designed to provide optimal imaging performance at a different fluorescence wavelength. In some embodiments, the system comprises a single objective lens, four tube lenses, and four image sensors, and each of the four tube lenses is designed to provide optimal imaging performance at a different fluorescence wavelength. In some embodiments, the design of the objective lens or the at least one tube lens is configured to optimize the modulation transfer function in the mid to high spatial frequency range. In some embodiments, the imaging performance metric comprises a measurement of modulation transfer function (MTF) at one or more specified spatial frequencies, defocus, spherical aberration, chromatic aberration, coma, astigmatism, field curvature, image distortion, contrast-to-noise ratio (CNR), or any combination thereof. In some embodiments, the difference in the imaging performance metric for imaging the upper interior surface and the lower interior surface of

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the flow cell is less than 10%. In some embodiments, the difference in imaging performance metric for imaging the upper interior surface and the lower interior surface of the flow cell is less than 5%. In some embodiments, the use of the at least one tube lens provides for an at least equivalent or better improvement in the imaging performance metric for dual-side imaging compared to that for a conventional system comprising an objective lens, a motion-actuated compensator, and an image sensor. In some embodiments, the use of the at least one tube lens provides for an at least 10% improvement in the imaging performance metric for dual-side imaging compared to that for a conventional system comprising an objective lens, a motion-actuated compensator, and an image sensor.

Disclosed herein are illumination systems for use in imaging-based solid-phase genotyping and sequencing applications, the illumination system comprising: a) a light source; and b) a liquid light-guide configured to collect light emitted by the light source and deliver it to a specified field-of-illumination on a support surface comprising tethered biological macromolecules.

In some embodiments, the illumination system further comprises a condenser lens. In some embodiments, the specified field-of-illumination has an area of at least 2 mm². In some embodiments, the light delivered to the specified field-of-illumination is of uniform intensity across a specified field-of-view for an imaging system used to acquire images of the support surface. In some embodiments, the specified field-of-view has an area of at least 2 mm². In some embodiments, the light delivered to the specified field-of-illumination is of uniform intensity across the specified field-of-view when a coefficient of variation (CV) for light intensity is less than 10%. In some embodiments, the light delivered to the specified field-of-illumination is of uniform intensity across the specified field-of-view when a coefficient of variation (CV) for light intensity is less than 5%. In some embodiments, the light delivered to the specified field-of-illumination has a speckle contrast value of less than 0.1. In some embodiments, the light delivered to the specified field-of-illumination has a speckle contrast value of less than 0.05.

INCORPORATION BY REFERENCE

All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference in its entirety. In the event of a conflict between a term herein and a term in an incorporated reference, the term herein controls.

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

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FIGS. 1A-1B schematically illustrate non-limiting examples of imaging dual surface support structures for presenting sample sites for imaging by the imaging systems disclosed herein. FIG. 1A: illustration of imaging front and rear interior surfaces of a flow cell. FIG. 1B: illustration of imaging front and rear exterior surfaces of a substrate.

FIGS. 2A-2B illustrate a non-limiting example of a multi-channel fluorescence imaging module comprising a dichroic beam splitter for transmitting an excitation light beam to a sample, and for receiving and redirecting by reflection the resultant fluorescence emission to four detection channels configured for detection of fluorescence emission at four different respective wavelengths or wavelength bands. FIG. 2A: top isometric view. FIG. 2B: bottom isometric view.

FIGS. 3A-3B illustrate the optical paths within the multi-channel fluorescence imaging module of FIGS. 2A and 2B comprising a dichroic beam splitter for transmitting an excitation light beam to a sample, and for receiving and redirecting by reflection a resultant fluorescence emission to four detection channels for detection of fluorescence emission at four different respective wavelengths or wavelength bands. FIG. 3A: top view. FIG. 3B: side view.

FIG. 4 is a graph illustrating a relationship between dichroic filter performance and beam angle of incidence.

FIG. 5 is a graph illustrating a relationship between beam footprint size and beam angle of incidence on a dichroic filter.

FIGS. 6A-6B schematically illustrate an example configuration of dichroic filters and detection channels of a multi-channel fluorescence imaging module wherein the dichroic filters have reflective surface tilted such that the angle between the incident beam (e.g., the central angle) and the reflective surface of the dichroic filter is less than 45°. FIG. 6A: schematic illustration of a multichannel fluorescence imaging module comprising four detection channels. FIG. 6B: detail view illustrating the angle of incidence (AOI) of a light beam on a dichroic reflector.

FIG. 7 provides a graph illustrating improved dichroic filter performance corresponding to the imaging module configuration illustrated in FIGS. 6A and 6B.

FIG. 8 provides a graph illustrating improved dichroic filter performance corresponding to the imaging module configuration illustrated in FIGS. 6A and 6B.

FIGS. 9A-9B provide graphs illustrating reduced surface deformation resulting from the imaging module configuration of FIGS. 6A and 6B. FIG. 9A illustrates the effect of folding angle on image quality degradation induced by the addition of 1 wave of PV spherical power to the last mirror. FIG. 9B illustrates the effect of folding angle on image quality degradation induced by the addition of 0.1 wave of PV spherical power to the last mirror.

FIGS. 10A-10B provide graphs illustrating improved excitation filter performance (e.g. sharper transitions between pass bands and surrounding stop bands) resulting from use of s-polarization of the excitation beam. FIG. 10A: transmission spectra for an example bandpass dichroic filter at angles of incidence of 40 degrees and 45 degrees, where the incident beam is linearly polarized and is p-polarized with respect to the plane of the dichroic filter. FIG. 10B: changing the orientation of the light source with respect to the dichroic filter, such that the incident beam is s-polarized with respect to the plane of the dichroic filter, results in a substantially sharper edge between the passband and the stopband.

FIGS. 11A-11B illustrate the modulation transfer function (MTF) of an example dual surface imaging system disclosed

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herein having a numerical aperture (NA) of 0.3. FIG. 11A: first surface. FIG. 11B: second surface.

FIGS. 12A-12B illustrate the MTF of an example dual surface imaging system disclosed herein having an NA of 0.4. FIG. 12A: first surface. FIG. 12B: second surface.

FIGS. 13A-13B illustrate the MTF of an example dual surface imaging system disclosed herein having an NA of 0.5. FIG. 13A: first surface. FIG. 13B: second surface.

FIGS. 14A-14B illustrate the MTF of an example dual surface imaging system disclosed herein having an NA of 0.6. FIG. 14A: first surface. FIG. 14B: second surface.

FIGS. 15A-15B illustrate the MTF of an example dual surface imaging system disclosed herein having an NA of 0.7. FIG. 15A: first surface. FIG. 15B: second surface.

FIGS. 16A-16B illustrate the MTF of an example dual surface imaging system disclosed herein having an NA of 0.8. FIG. 16A: first surface. FIG. 16B: second surface.

FIGS. 17A-17B provide plots of the calculated Strehl ratio for imaging a second flow cell surface through a first flow cell surface. FIG. 17A: plot of the Strehl ratios for imaging a second flow cell surface through a first flow cell surface as a function of the thickness of the intervening fluid layer (fluid channel height) for different objective lens and/or optical system numerical apertures. FIG. 17B: plot of the Strehl ratio as a function of numerical aperture for imaging a second flow cell surface through a first flow cell surface and an intervening layer of water having a thickness of 0.1 mm.

FIG. 18 provides a schematic illustration of a dual-wavelength excitation/four channel emission fluorescence imaging system of the present disclosure.

FIG. 19 provides an optical ray tracing diagram for an objective lens design that has been designed for imaging a surface on the opposite side of a 0.17 mm thick coverslip.

FIG. 20 provides a plot of the modulation transfer function for the objective lens illustrated in FIG. 19 as a function of spatial frequency when used to image a surface on the opposite side of a 0.17 mm thick coverslip.

FIG. 21 provides a plot of the modulation transfer function for the objective lens illustrated in FIG. 19 as a function of spatial frequency when used to image a surface on the opposite side of a 0.3 mm thick coverslip.

FIG. 22 provides a plot of the modulation transfer function for the objective lens illustrated in FIG. 19 as a function of spatial frequency when used to image a surface that is separated from that on the opposite side of a 0.3 mm thick coverslip by a 0.1 mm thick layer of aqueous fluid.

FIG. 23 provides a plot of the modulation transfer function for the objective lens illustrated in FIG. 19 as a function of spatial frequency when used to image a surface on the opposite side of a 1.0 mm thick coverslip.

FIG. 24 provides a plot of the modulation transfer function for the objective lens illustrated in FIG. 19 as a function of spatial frequency when used to image a surface that is separated from that on the opposite side of a 1.0 mm thick coverslip by a 0.1 mm thick layer of aqueous fluid.

FIG. 25 provides a ray tracing diagram for a tube lens design which, if used in conjunction with the objective lens illustrated in FIG. 19, provides for improved dual-side imaging through a 1 mm thick coverslip.

FIG. 26 provides a plot of the modulation transfer function for the combination of objective lens and tube lens illustrated in FIG. 25 as a function of spatial frequency when used to image a surface on the opposite side of a 1.0 mm thick coverslip.

FIG. 27 provides a plot of the modulation transfer function for the combination of objective lens and tube lens

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illustrated in FIG. 25 as a function of spatial frequency when used to image a surface that is separated from that on the opposite side of a 1.0 mm thick coverslip by a 0.1 mm thick layer of aqueous fluid.

FIG. 28 provides ray tracing diagrams for tube lens design (left) of the present disclosure that has been optimized to provide high-quality, dual-side imaging performance. Because the tube lens is no longer infinity-corrected, an appropriately designed null lens (right) may be used in combination with the tube lens to compensate for the non-infinity-corrected tube lens for manufacturing and testing purposes.

FIG. 29 illustrates one non-limiting example of a single capillary flow cell having 2 fluidic adaptors.

FIG. 30 illustrates one non-limiting example of a flow cell cartridge comprising a chassis, fluidic adapters, and optionally other components, that is designed to hold two capillaries.

FIG. 31 illustrates one non-limiting example of a system comprising a single capillary flow cell connected to various fluid flow control components, where the single capillary is compatible with mounting on a microscope stage or in a custom imaging instrument for use in various imaging applications.

FIG. 32 illustrates one non-limiting example of a system that comprises a capillary flow cell cartridge having integrated diaphragm valves to reduce or minimize dead volume and conserve certain key reagents.

FIG. 33 illustrates one non-limiting example of a system that comprises a capillary flow cell, a microscope setup, and a temperature control mechanism.

FIG. 34 illustrates one non-limiting example for temperature control of the capillary flow cells through the use of a metal plate that is placed in contact with the flow cell cartridge.

FIG. 35 illustrates one non-limiting approach for temperature control of the capillary flow cells that comprises a non-contact thermal control mechanism.

FIGS. 36A-36C illustrates non-limiting examples of flow cell device fabrication. FIG. 36A shows the preparation of one-piece glass flow cell. FIG. 36B shows the preparation of two-piece glass flow cell. FIG. 36C shows the preparation of three-piece glass flow cell.

FIGS. 37A-37C illustrates non-limiting examples of glass flow cell designs. FIG. 37A shows a one-piece glass flow cell design. FIG. 37B shows a two-piece glass flow cell design.

FIG. 37C shows a three-piece glass flow cell design.

FIG. 38 illustrates visualization of cluster amplification in a capillary lumen.

FIG. 39 provides a non-limiting example of a block diagram for a sequencing system as disclosed herein.

FIG. 40 provides a non-limiting example of a flow chart for a sequencing method as disclosed herein.

FIG. 41 provides a non-limiting example of a schematic for a structured illumination system as disclosed herein.

FIG. 42 provides a non-limiting example of a flow chart for acquiring and processing structured illumination images of a flow cell surface as disclosed herein.

FIGS. 43A-43B provide non-limiting schematic illustrations of a multiplexed read-head as disclosed herein. FIG. 43A: side view of a multiplexed read-head in which individual microfluorimeters are configured to image a common surface, e.g., the interior surface of a flow cell. FIG. 43B: top view of a multiplexed read-head illustrating the imaging paths acquired by individual microfluorimeters of the multiplexed read-head.

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FIGS. 44A-44B provide non-limiting schematic illustrations of a multiplexed read-head as disclosed herein. FIG. 44A: side view of a multiplexed read-head in which a first subset of a plurality of individual microfluorimeters is configured to image a first surface, e.g., a first interior surface of a flow cell, and a second subset of the plurality of individual microfluorimeters is configured to image a second surface, e.g., a second interior surface of a flow cell. FIG. 44B: top view of the multiplexed read-head of FIG. 44A illustrating the imaging paths acquired by individual microfluorimeters of the multiplexed read-head.

DETAILED DESCRIPTION

There is a need for fluorescence imaging methods and systems that provide increased optical resolution and improved image quality for genomics applications that lead to corresponding improvements in genomic testing accuracy. Disclosed herein are optical system designs for high-performance fluorescence imaging methods and systems that may provide any one or more of improved optical resolution (including high performance optical resolution), improved image quality, and higher throughput for fluorescence imaging-based genomics applications. The disclosed optical illumination and imaging system designs may provide any one or more of the following advantages: improved dichroic filter performance, increased uniformity of dichroic filter frequency response, improved excitation beam filtering, larger fields-of-view, increased spatial resolution, improved modulation transfer, contrast-to-noise ratio, and image quality, higher spatial sampling frequency, faster transitions between image capture when repositioning the sample plane to capture a series of images (e.g., of different fields-of-view), improved imaging system duty cycle, and higher throughput image acquisition and analysis.

In some instances, improvements in imaging performance, e.g., for dual-side (flow cell) imaging applications comprising the use of thick flow cell walls (e.g., wall (or coverslip) thickness $>700\ \mu\text{m}$) and fluid channels (e.g., fluid channel height or thickness of $50\text{-}200\ \mu\text{m}$) may be achieved using novel objective lens designs that correct for optical aberration introduced by imaging surfaces on the opposite side of thick coverslips and/or fluid channels from the objective.

In some instances, improvements in imaging performance, e.g., for dual-side (flow cell) imaging applications comprising the use of thick flow cell walls (e.g., wall (or coverslip) thickness $>700\ \mu\text{m}$) and fluid channels (e.g., fluid channel height or thickness of $50\text{-}200\ \mu\text{m}$) may be achieved even when using commercially-available, off-the-shelf objectives by using a novel tube lens design that, unlike the tube lens in a conventional microscope that simply forms an image at the intermediate image plane, corrects for the optical aberrations induced by the thick flow cell walls and/or intervening fluid layer in combination with the objective.

In some instances, improvements in imaging performance, e.g., for multichannel (e.g., two-color or four-color) imaging applications, may be achieved by using multiple tube lenses, one for each imaging channel, where each tube lens design has been optimized for the specific wavelength range used in that imaging channel.

In some instances, improvements in imaging performance, e.g., for dual-side (flow cell) imaging applications, may be achieved by using an electro-optical phase plate in combination with an objective lens to compensate for the optical aberrations induced by the layer of fluid separating

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the upper (near) and lower (far) interior surfaces of a flow cell. In some instances, this design approach may also compensate for vibrations introduced by, e.g., a motion-actuated compensator that is moved in or out of the optical path depending on which surface of the flow cell is being imaged.

Various multichannel fluorescence imaging module designs are disclosed that may include illumination and imaging optical paths comprising folded optical paths (e.g., comprising one or more beam splitters or beam combiners, such as dichroic beam splitters or combiners) that direct an excitation light beam to an objective lens, and direct emission light transmitted through the objective lens to a plurality of detection channels. Some particularly advantageous features of the fluorescence imaging modules described herein include specification of dichroic filter incidence angles that result in sharper and/or more uniform transitions between passband and stopband wavelength regions of the dichroic filters. Such filters may be included within the folded optics and may comprise dichroic beam splitters or combiners. Further advantageous features of the disclosed imaging optics designs may include the position and orientation of one or more excitation light sources and one or more detection optical paths with respect to the objective lens and to a dichroic filter that receives the excitation beam. The excitation beam may also be linearly-polarized and the orientation of the linear polarization may be such that s-polarized light is incident on the dichroic reflective surface of the dichroic filter. Such features may potentially improve excitation beam filtering and/or reduce wavefront error introduced into the emission light beam due to surface deformation of dichroic filters. The fluorescence imaging modules described herein may or may not include any of these features and may or may not include any of these advantages.

Also described herein are devices and systems configured to analyze large numbers of different nucleic acid sequences by imaging, e.g., arrays of immobilized nucleic acid molecules or amplified nucleic acid clusters formed on flow cell surfaces. The devices and systems described herein can also be useful in, e.g., performing sequencing for comparative genomics, tracking gene expression, performing micro RNA sequence analysis, epigenomics, aptamer and phage display library characterization, and for performing other sequencing applications. The devices and systems disclosed herein comprise various combinations of optical, mechanical, fluidic, thermal, electrical, and computing devices/aspects. The advantages conferred by the disclosed flow cell devices, cartridges, and systems include, but are not limited to: (i) reduced device and system manufacturing complexity and cost, (ii) significantly lower consumable costs (e.g., as compared to those for currently available nucleic acid sequencing systems), (iii) compatibility with typical flow cell surface functionalization methods, (iv) flexible flow control when combined with microfluidic components, e.g., syringe pumps and diaphragm valves, etc., and (v) flexible system throughput.

Disclosed herein are capillary flow-cell devices and capillary flow cell cartridges that are constructed from off-the-shelf, disposable, single lumen (e.g., single fluid flow channel) or multi-lumen capillaries that may also comprise fluidic adaptors, cartridge chassis, one or more integrated fluid flow control components, or any combination thereof. Also disclosed herein are capillary flow cell-based systems that may comprise one or more capillary flow cell devices (or microfluidic chips), one or more capillary flow cell

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cartridges (or microfluidic cartridges), fluid flow controller modules, temperature control modules, imaging modules, or any combination thereof.

The design features of some disclosed capillary flow cell devices, cartridges, and systems include, but are not limited to, (i) unitary flow channel construction, (ii) sealed, reliable, and repetitive switching between reagent flows that can be implemented with a simple load/unload mechanism such that fluidic interfaces between the system and capillaries are reliably sealed, thereby facilitating capillary replacement and system reuse, and enabling precise control of reaction conditions such as reagent concentration, pH, and temperature, (iii) replaceable single fluid flow channel devices or capillary flow cell cartridges comprising multiple flow channels that can be used interchangeably to provide flexible system throughput, and (iv) compatibility with a wide variety of detection methods such as fluorescence imaging.

Although the disclosed capillary flow cell devices and systems, capillary flow cell cartridges, capillary flow cell-based systems, microfluidic devices and cartridges, and microfluidic chip-based systems, are described primarily in the context of their use for nucleic acid sequencing applications, various aspects of the disclosed devices and systems may be applied not only to nucleic acid sequencing but also to any other type of chemical analysis, biochemical analysis, nucleic acid analysis, cell analysis, or tissue analysis application. It shall be understood that different aspects of the disclosed methods, devices, and systems can be appreciated individually, collectively, or in combination with each other. Although discussed herein primarily in the context of fluorescence imaging (including, e.g., fluorescence microscopy imaging, fluorescence confocal imaging, two-photon fluorescence, and the like), it will be understood by those of skill in the art that many of the disclosed optical design approaches and features are applicable to other imaging modes, e.g., bright-field imaging, dark-field imaging, phase contrast imaging, and the like.

Definitions

Unless otherwise defined, all of the technical terms used herein have the same meaning as commonly understood by one of ordinary skill in the art in the field to which this disclosure belongs.

As used in this specification and the appended claims, the singular forms “a”, “an”, and “the” include plural references unless the context clearly dictates otherwise. Any reference to “or” herein is intended to encompass “and/or” unless otherwise stated.

As used herein, the term ‘about’ a number refers to that number plus or minus 10% of that number. The term ‘about’ when used in the context of a range refers to that range minus 10% of its lowest value and plus 10% of its greatest value.

As used herein, the phrases “imaging module”, “imaging unit”, “imaging system”, “optical imaging module”, “optical imaging unit”, and “optical imaging system” are used interchangeably, and may comprise components or sub-systems of a larger system that may also include, e.g., fluidics modules, temperature control modules, translation stages, robotic fluid dispensing and/or microplate handling, processor or computers, instrument control software, data analysis and display software, etc.

As used herein, the term “detection channel” refers to an optical path (and/or the optical components therein) within an optical system that is configured to deliver an optical signal arising from a sample to a detector. In some instances,

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a detection channel may be configured for performing spectroscopic measurements, e.g., monitoring a fluorescence signal or other optical signal using a detector such as a photomultiplier. In some instances, a “detection channel” may be an “imaging channel”, i.e., an optical path (and/or the optical components therein) within an optical system that is configured to capture and deliver an image to an image sensor.

As used herein, a “detectable label” may refer to any of a variety of detectable labels or tags known to those of skill in the art. Examples include, but are not limited to, chromophores, fluorophores, quantum dots, upconverting phosphors, luminescent or chemiluminescent molecules, radioisotopes, magnetic nanoparticles, mass tags, and the like. In some instances, a preferred label may comprise a fluorophore.

As used herein, the term “excitation wavelength” refers to the wavelength of light used to excite a fluorescent indicator (e.g., a fluorophore or dye molecule) and generate fluorescence. Although the excitation wavelength is typically specified as a single wavelength, e.g., 620 nm, it will be understood by those of skill in the art that this specification refers to a wavelength range or excitation filter bandpass that is centered on the specified wavelength. For example, in some instances, light of the specified excitation wavelength comprises light of the specified wavelength ± 2 nm, ± 5 nm, ± 10 nm, ± 20 nm, ± 40 nm, ± 80 nm, or more. In some instances, the excitation wavelength used may or may not coincide with the absorption peak maximum of the fluorescent indicator.

As used herein, the term “emission wavelength” refers to the wavelength of light emitted by a fluorescent indicator (e.g., a fluorophore or dye molecule) upon excitation by light of an appropriate wavelength. Although the emission wavelength is typically specified as a single wavelength, e.g., 670 nm, it will be understood by those of skill in the art that this specification refers to a wavelength range or emission filter bandpass that is centered on the specified wavelength. In some instances, light of the specified emission wavelength comprises light of the specified wavelength ± 2 nm, ± 5 nm, ± 10 nm, ± 20 nm, ± 40 nm, ± 80 nm, or more. In some instances, the emission wavelength used may or may not coincide with the emission peak maximum of the fluorescent indicator.

As used herein, fluorescence is ‘specific’ if it arises from fluorophores that are annealed or otherwise tethered to the surface, such as fluorescently labeled nucleic acid sequences having a region of reverse complementarity to a corresponding segment of an oligonucleotide adapter on the surface and annealed to said corresponding segment. This fluorescence is contrasted with fluorescence arising from fluorophores not tethered to the surface through such an annealing process, or in some cases to background fluorescence of the surface.

As used herein, a “nucleic acid” (also referred to as a “nucleic acid molecule”, a “polynucleotide”, “oligonucleotide”, ribonucleic acid (RNA), or deoxyribonucleic acid (DNA)) is a linear polymer of two or more nucleotides joined by covalent internucleosidic linkages, or variants or functional fragments thereof. In naturally occurring examples of nucleic acids, the internucleoside linkage is typically a phosphodiester bond. However, other examples optionally comprise other internucleoside linkages, such as phosphorothiolate linkages and may or may not comprise a phosphate group. Nucleic acids include double- and single-stranded DNA, as well as double- and single-stranded RNA, DNA/RNA hybrids, peptide-nucleic acids (PNAs), hybrids

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between PNAs and DNA or RNA, and may also include other types of nucleic acid modifications.

As used herein, a “nucleotide” refers to a nucleotide, nucleoside, or analog thereof. In some cases, the nucleotide is an N- or C-glycoside of a purine or pyrimidine base (e.g., a deoxyribonucleoside containing 2-deoxy-D-ribose or ribonucleoside containing D-ribose). Examples of other nucleotide analogs include, but are not limited to, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, and the like.

Fluorescence Imaging Viewed as an Information Pipeline:

A useful abstraction of the role that fluorescence imaging systems plays in typical genomic assay techniques (including nucleic acid sequencing applications) is as an information pipeline, where the photon signal enters at one end of the pipeline, e.g., the objective lens used for imaging, and location specific information regarding the fluorescence signal emerges at the other end of the pipeline, e.g., at the position of the image sensor. When more information is pumped through this pipeline, some content, inevitably, will be lost during this transfer process and never recovered. An example of this case is when too many labeled molecules (or clonally-amplified clusters of molecules) are present within a small region of a substrate surface to be clearly resolved in the image; at the position of the image sensor, it becomes difficult to differentiate photon signals arising from adjacent clusters of molecules, thus increasing the probability of attributing the signal to the wrong cluster and leading to detection errors.

Design of Optical Imaging Modules:

The goal of designing an optical imaging module is thus to maximize the flow of information content through this detection pipeline and to minimize detection errors. Several key design elements need to be addressed in the design process, including:

1) Matching the physical feature density on the substrate surface to be imaged with the overall image quality of the optical imaging system and the pixel sampling frequency of the image sensor used. A mismatch of these parameters may result in loss of information or sometimes even the generation of false information, e.g., spatial aliasing may arise when pixel sampling frequency is lower than twice the optical resolution limit.

2) Matching the size of the area to be imaged with the overall image quality of the optical imaging system and focus quality across the entire field of view.

3) Matching the optical collection efficiency, modulation transfer function, and image sensor performance characteristics of the optical system design with the fluorescence photon flux expected for the input excitation photon flux, dye efficiency (related to dye extinction coefficient and fluorescence quantum yield), while accounting for background signal and system noise characteristics.

4) Maximizing the separation of spectral content to reduce cross talk between fluorescence imaging channels.

5) Effective synchronization of image acquisition steps with repositioning of the sample or optics between image capture of different fields-of-view to minimize the down time (or maximize the duty cycle) of the imaging system and thus maximize the overall throughput of the image capture process.

This disclosure describes a systematic way to address each of the design elements outlined above and to create component level specifications for the imaging system.

Improved Optical Resolution and Image Quality to Improve or Maximize Information Transfer and Throughput:

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One non-limiting design practice may be to start with the optical resolution required to distinguish two adjacent features as specified in terms of a number, X, of line pairs per mm (lp/mm) and translate it to a corresponding numerical aperture (NA) requirement. The numerical aperture requirement can then be used to assess the resulting impact on modulation transfer function and image contrast.

The standard modulation transfer function (MTF) describes the spatial frequency response for image contrast (modulation) transferred through an optical system; image contrast decreases as a function of spatial frequency and increases with increasing NA. This function limits the contrast/modulation that can be achieved for a given NA. Furthermore, wave front error can negatively impact the MTF, thus making it desirable to improve or optimize the optical system design using the true system MTF instead of that predicted by diffraction-limited optics. Note that, as used herein, MTF will refer to the total system MTF (including the complete optical path from coverslip to image sensor) although design practice may primarily consider the MTF of the objective lens.

In genomic testing applications, where the target to be imaged is an array of high density “spots” on a surface (either randomly distributed or patterned), one can determine the minimum modulation transfer value required by downstream analysis to resolve two adjacent spots and discriminate between four possible states (e.g., ON-OFF, ON-ON, OFF-ON and OFF-OFF). For example, assume that the spots are small enough to be approximated as point sources of light. Assuming that the detection task is to determine if the two adjacent spots separated by a distance, d, are ON or OFF (in other words, bright or dark), and that the contrast-to-noise ratio (CNR) for the fluorescence signals arising from the spots at the sample plane (or object plane) is C_{sample} , then under ideal conditions the CNR of the readout signal for the two adjacent spots at the image sensor plane, C_{image} , can be closely approximated as $C_{image} = C_{sample} * MTF(1/d)$, where $MTF(1/d)$ is the MTF value at spatial frequency $= (1/d)$.

In a typical design, the value of C may need to be at least 4 so that a simple threshold method can be used to avoid misclassification of fluorescence signals. Assuming a Gaussian distribution of fluorescence signal intensities around a mean value, at $C_{image} > 4$, the expected error in correctly classifying fluorescence signals (e.g., as being ON or OFF) is $< 0.035\%$. The use of proprietary high CNR sequencing and surface chemistry, such as that described in U.S. patent application Ser. No. 16/363,842, allows one to achieve sample plane CNR (C_{sample}) values for clusters of clonally-amplified, labeled oligonucleotide molecules tethered to a substrate surface of greater than 12 (or even much higher) when measured for a sparse field (i.e., at a low surface density of clusters or spots) where the MTF has a value of close to 100%. Assuming a sample plane CNR value of $C_{sample} > 12$ and targeting a classification error rate of $< 0.1\%$ (thus, $C_{image} > 4$), in some implementations the minimum value for $M(1/d)$ can be determined as $M(1/d) = 4/12 \sim 33\%$. Thus, a modulation transfer function threshold of at least 33% may be used to retain the information content of the transferred image.

Design practice can relate the minimum separation distance of two features or spots, d, to the optical resolution requirement (specified as noted above in terms of X (lp/mm)) as $d = (1 \text{ mm})/X$, i.e., d is the minimum separation distance between two features or spots which can be fully resolved by the optical system. In some designs disclosed herein, where the objective of the design analysis is to

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increase or maximize relevant information transfer, this design criterion can be relaxed to $d=(1\text{ mm})/X/A$, where $2>A>1$. For the same optical resolution of $X\text{ lp/mm}$, the value of d , the minimum resolvable spot separation distance at the sample plane, is reduced, thereby enabling the use of higher feature densities.

Design practice determines the minimum spatial sampling frequency at the sample plane using the Nyquist criteria, where spatial sampling frequency $S\geq 2*X$ (and where X is the optical resolution of the imaging system specified in terms of $X\text{ lp/mm}$ as noted above). When the system spatial sampling frequency is close to the Nyquist criteria, as is often the case, imaging system resolution of greater than S results in aliasing as the higher frequency information resolved by the optical system cannot be sufficiently sampled by the image sensor.

In the some of the designs disclosed herein, an oversampling scheme based on the relationship $S=B*Y$ (where $B\geq 2$ and Y is the true optical system MTF limit) may be used to further improve the information transfer capacity of the imaging system. As indicated above, $X\text{ (lp/mm)}$ corresponds to a practical, non-zero ($>33\%$) minimum modulation transfer value, whereas $Y\text{ (lp/mm)}$ is the limit of optical resolution so modulation at $Y\text{ (lp/mm)}$ is 0. Thus, in the disclosed designs, $Y\text{ (lp/mm)}$ may advantageously be significantly greater than X . For values of $B\geq 2$, the disclosed designs are oversampling for the sample object frequency X , i.e., $S\geq B*Y>2*X$.

The above relationship can be used to determine the system magnification and may provide an upper bound for image sensor pixel size. The choice of image sensor pixel size is matched to the system optical quality as well to the spatial sampling frequency required to reduce aliasing. The lower bound of image sensor pixel size can be determined based on photon throughput, as relative noise contributions increase with smaller pixels.

Other design approaches are, however, also possible. For example, reducing the NA to less than 0.6 (e.g., 0.5 or less) may provide increased depth of field. Such increased depth of field may enable dual surface imaging wherein two surfaces at different depths can be imaged at the same time with or without refocusing. As discussed above, reducing NA may reduce optical resolution. In some implementations, use of higher excitation beam power, e.g., 1 W or higher, may be employed to produce strong signal. An inherently high contrast sample (i.e., comprising a sample surface that exhibits strong foreground signal and dramatically reduced background signal, may also be used to facilitate acquisition of high contrast-to-noise ratio (CNR) images, e.g., having CNR values of >20 , that provide for improved signal discrimination for base-calling in nucleic acid sequencing applications, etc. In some optical system designs disclosed herein, sample support structures such as flow cells having hydrophilic surfaces are used to reduce background noise.

In various implementations, a large field-of-view (FOV) is provided by the disclosed optical systems. For example, a FOV of greater than 2 or 3 mm may be provided with some optical imaging systems comprising, e.g., an objective lens and a tube lens. In some cases, the optical imaging system provides a reduced magnification, for example, a magnification of less than $10\times$. Such reduced magnification may in some implementations facilitate large FOV designs. Despite a reduced magnification, the optical resolution of such systems can still be sufficient as detector arrays having small pixel size or pitch may be used. In some implementations, image sensors comprising a pixel size that is smaller than

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twice the optical resolution provided by the optical imaging system (e.g., objective and tube lens) may be used to satisfy the Nyquist theorem.

Still other designs are also possible. In some optical designs configured to provide for dual surface imaging where two surfaces at different depths can be imaged at the same time, the optical imaging system (e.g., the objective lens and/or tube lens) is configured to reduce optical aberration for imaging said two surfaces (e.g., two planes) at those two respective depths more than at other locations (e.g., other planes) at other depths. Additionally, the optical imaging system may be configured to reduce aberration for imaging said two surfaces (e.g., two planes) at those two respective depths through a transmissive layer on said sample support structure (such as a layer of glass (e.g., a cover slip) and through a solution (e.g., an aqueous solution) comprising the sample or in contact with a sample on at least one of said two surfaces.

Multichannel Fluorescence Imaging Modules and Systems:

In some instances, the imaging modules or systems disclosed herein may comprise fluorescence imaging modules or systems. In some instances, the fluorescence imaging systems disclosed herein may comprise a single fluorescence excitation light source (for providing excitation light at a single wavelength or within a single excitation wavelength range) and an optical path configured to deliver the excitation light to a sample (e.g., fluorescently-tagged nucleic acid molecules or clusters thereof disposed on a substrate surface). In some instances, the fluorescence imaging systems disclosed herein may comprise a single fluorescence emission imaging and detection channel, e.g., an optical path configured to collect fluorescence emitted by the sample and deliver an image of the sample (e.g., an image of a substrate surface on which fluorescently-tagged nucleic acid molecules or clusters thereof are disposed) to an image sensor or other photodetection device. In some instances, the fluorescence imaging systems may comprise two, three, four, or more than four fluorescence excitation light sources and/or optical paths configured to deliver excitation light at two, three, four, or more than four excitation wavelengths (or within two, three, four, or more than four excitation wavelength ranges). In some instances, the fluorescence imaging systems disclosed herein may comprise two, three, four, or more than four fluorescence emission imaging and detection channels configured to collect fluorescence emitted by the sample at two, three, four, or more than four emission wavelengths (or within two, three, four, or more than four emission wavelength ranges and deliver an image of the sample (e.g., an image of a substrate surface on which fluorescently-tagged nucleic acid molecules or clusters thereof are disposed) to two, three, four, or more than four image sensors or other photodetection devices.

Dual Surface Imaging:

In some instances, the imaging systems disclosed herein, including fluorescence imaging systems, may be configured to acquire high-resolution images of a single sample support structure or substrate surface. In some instances, the imaging systems disclosed herein, including fluorescence imaging systems, may be configured to acquire high-resolution images of two or more sample support structures or substrate surfaces, e.g., two or more surfaces of a flow cell. In some instances, the high-resolution images provided by the disclosed imaging systems may be used to monitor reactions occurring on the two or more surfaces of the flow cell (e.g., nucleic acid hybridization, amplification, and/or sequencing reactions) as various reagents flow through the flow cell or

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around a flow cell substrate. FIG. 1A and FIG. 1B provide schematic illustrations of such dual surface support structures. FIG. 1A shows a dual surface support structure such as a flow cell that includes an internal flow channel through which an analyte or reagent can be flowed. The flow channel may be formed between first and second, top and bottom, and/or front and back layers such as first and second, top and bottom, and/or front and back plates as shown. One or more of the plates may include a glass plate, such as a coverslip, or the like. In some implementations, the layer comprises borosilicate glass, quartz, or plastic. Interior surfaces of these top and bottom layers provide walls of the flow channel that assist in confining the flow of analyte or reagent through the flow channel of the flow cell. In some designs, these interior surfaces are planar. Similarly, the top and bottom layers may be planar. In some designs, at least one additional layer (not shown) is disposed between the top and bottom layers. This additional layer may have one or more pathways cut therein that assist in defining one or more flow channels and controlling the flow of the analyte or reagent within the flow channel. Additional discussion of sample support structures, e.g., flow cells, can be found below.

FIG. 1A schematically illustrates a plurality of fluorescing sample sites on the first and second, top and bottom, and/or front and back interior surfaces of the flow cell. In some implementations, reactions may occur at these sites to bind sample such that fluorescence is emitted from these sites (note that FIG. 1A is schematic and not drawn to scale; for example, the size and spacing of the fluorescing sample sites may be smaller than shown).

FIG. 1B shows another dual surface support structure having two surfaces containing fluorescing sample sites to be imaged. The sample support structure comprises a substrate having first and second, top and bottom, and/or front and back exterior surfaces. In some designs, these exterior surfaces are planar. In various implementations, the analyte or reagent is flowed across these first and second exterior surfaces. FIG. 1B schematically illustrates a plurality of fluorescing sample sites on the first and second, top and bottom, and/or front and back exterior surfaces of the sample support structure. In some implementations, reactions may occur at these sites to bind sample such that fluorescence is emitted from these sites (note that FIG. 1B is schematic and not drawn to scale; for example, the size and spacing of the fluorescing sample sites may be smaller than shown).

In some instances, the fluorescence imaging modules and systems described herein may be configured to image such fluorescing sample sites on first and second surfaces at different distances from the objective lens. In some designs, only one of the first or second surfaces is in focus at a time. Accordingly, in such designs, one of the surfaces is imaged at a first time, and the other surface is imaged at a second time. The focus of the fluorescence imaging module may be changed after imaging one of the surfaces in order to image the other surface with comparable optical resolution, as the images of the two surfaces are not simultaneously in focus. In some designs, an optical compensation element may be introduced into the optical path between the sample support structure and the image sensor in order to image one of the two surfaces. The depth of field in such fluorescence imaging configurations may not be sufficiently large to include both the first and second surfaces. In some implementations of the fluorescence imaging modules described herein, both the first and second surfaces may be imaged at the same time, i.e., simultaneously. For example, the fluorescence imaging module may have a depth of field that is sufficiently

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large to include both surfaces. In some instances, this increased depth of field may be provided by, for example, reducing the numerical aperture of the objective lens (or microscope objective) as will be discussed in more detail below.

As shown in FIGS. 1A and 1B, the imaging optics (e.g., an objective lens) may be positioned at a suitable distance (e.g., a distance corresponding to the working distance) from the first and second surfaces to form in-focus images of the first and second surfaces on an image sensor of a detection channel. As shown in the example of FIGS. 1A and 1B, the first surface may be between said objective lens and the second surface. For example, as illustrated, the objective lens is disposed above both the first and second surfaces, and the first surface is disposed above the second surface. The first and second surfaces, for example, are at different depths. The first and second surfaces are at different distances from any one or more of the fluorescence imaging module, the illumination and imaging module, imaging optics, or the objective lens. The first and second surfaces are separated from each other with the first surface spaced apart above the second surface. In the example shown, the first and second surfaces are planar surfaces and are separated from each other along a direction normal to said first and second planar surfaces. Also, in the example shown, said objective lens has an optical axis and said first and second surfaces are separated from each other along the direction of said optical axis. Similarly, the separation between the first and second surfaces may correspond to the longitudinal distance such as along the optical path of the excitation beam and/or along an optical axis through the fluorescence imaging module and/or the objective lens. Accordingly, these two surfaces may be separated by a distance from each other in the longitudinal (Z) direction, which may be along the direction of the central axis of the excitation beam and/or the optical axis of the objective lens and/or the fluorescence imaging module. This separation may correspond, for example, to a flow channel within a flow cell in some implementations.

In various designs, the objective lens (possibly in combination with another optical component, e.g., a tube lens) have a depth of field and/or depth of focus that is at least as large as the longitudinal separation (in the Z direction) between the first and second surfaces. The objective lens, alone or in combination with the additional optical component, may thus simultaneously form in-focus images of both the first and the second surface on an image sensor of one or more detection channels where these images have comparable optical resolution. In some implementations, the imaging module may or may not need to be re-focused to capture images of both the first and second surfaces with comparable optical resolution. In some implementations, compensation optics need not be moved into or out of an optical path of the imaging module to form in-focus images of the first and second surfaces. Similarly, in some implementations, one or more optical elements (e.g., lens elements) in the imaging module (e.g., the objective lens and/or a tube lens) need not be moved, for example, in the longitudinal direction along the first and/or second optical paths (e.g., along the optical axis of the imaging optics) to form in-focus images of the first surface in comparison to the location of said one or more optical element when used to form in-focus images of the second surface. In some implementations, however, the imaging module includes an autofocus system configured to provide both the first and second surface in focus at the same time. In various implementations, the sample is in focus to sufficiently resolve the sample sites, which are closely

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spaced together in lateral directions (e.g., the X and Y directions). Accordingly, in various implementations, no optical element enters an optical path between the sample support structure (e.g., between a translation stage that supports the sample support structure) and an image sensor (or photodetector array) in the at least one detection channel in order to form in-focus images of fluorescing sample sites on a first surface of the sample support structure and on a second surface of said sample support structure. Similarly, in various implementations, no optical compensation is used to form an in-focus image of fluorescing sample sites on a first surface of the sample support structure on the image sensor or photodetector array that is not identical to optical compensation used to form an in-focus image of fluorescing sample sites on a second surface of the sample support structure on the image sensor or photodetector array. Additionally, in certain implementations, no optical element in an optical path between the sample support structure (e.g., between a translation stage that supports the sample support structure) and an image sensor in the at least one detection channel is adjusted differently to form an in-focus image of fluorescing sample sites on a first surface of the sample support structure than to form an in-focus image of fluorescing sample sites on a second surface of the sample support structure. Similarly, in some various implementations, no optical element in an optical path between the sample support structure (e.g., between a translation stage that supports the sample support structure) and an image sensor in the at least one detection channel is moved a different amount or a different direction to form an in-focus image of fluorescing sample sites on the a first surface of the sample support structure on the image sensor than to form an in-focus image of fluorescing sample sites on a second surface of said sample support structure on the image sensor. Any combination of the features is possible. For example, in some implementations, in-focus images of the upper interior surface and the lower interior surface of the flow cell can be obtained without moving an optical compensator into or out of an optical path between the flow cell and the at least one image sensor and without moving one or more optical elements of the imaging system (e.g., the objective and/or tube lens) along the optical path (e.g., optical axis) therebetween. For example, in-focus images of the upper interior surface and the lower interior surface of the flow cell can be obtained without moving one or more optical elements of the tube lens into or out of the optical path, or without moving one or more optical elements of the tube lens along the optical path (e.g., optical axis) therebetween.

Any one or more of the fluorescence imaging module, the illumination optical path, the imaging optical path, the objective lens, or the tube lens may be designed to reduce or minimize optical aberration at two locations such as two planes corresponding to two surfaces on a flow cell or other sample support structure, for example, where fluorescing sample sites are located. Any one or more of the fluorescence imaging module, the illumination optical path, the imaging optical path, the objective lens, or the tube lens may be designed to reduce or minimize optical aberration at the selected locations or planes relative to other locations or planes, such as first and second surfaces containing fluorescing sample sites on a dual surface flow cell. For example, any one or more of the fluorescence imaging module, the illumination optical path, the imaging optical path, the objective lens, or the tube lens may be designed to reduce or minimize optical aberration at two depths or planes located at different distances from the objective lens as compared to the aberrations associated with other depths

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or planes at other distances from the objective lens. For example, optical aberration may be less for imaging the first and second surfaces than elsewhere in a region ranging from about 1 to about 10 mm from the objective lens. Additionally, any one or more of the fluorescence imaging module, the illumination optical path, the imaging optical path, the objective lens, or the tube lens may, in some instances, be configured to compensate for optical aberration induced by transmission of emission light through one or more portions of the sample support structure such as a layer that includes one of the surfaces on which sample adheres as well as possibly a solution that is in contact with the sample. This layer (e.g., a coverslip or the wall of a flow cell) may comprise, e.g., glass, quartz, plastic, or other transparent material having a refractive index and that introduces optical aberration.

Accordingly, the imaging performance may be substantially the same when imaging the first surface and second surface. For example, the optical transfer functions (OTF) and/or modulation transfer functions (MTF) may be the substantially the same for imaging of the first and second surfaces. Either or both of these transfer functions may, for example, be within 20%, within 15%, within 10%, within 5%, within 2.5%, or within 1% of each other, or within any range formed by any of these values at one or more specified spatial frequencies or when averaged over a range of spatial frequencies. Accordingly, an imaging performance metric may be substantially the same for imaging the upper interior surface or the lower interior surface of the flow cell without moving an optical compensator into or out of an optical path between the flow cell and the at least one image sensor, and without moving one or more optical elements of the imaging system (e.g., the objective and/or tube lens) along the optical path (e.g., optical axis) therebetween. For example, an imaging performance metric may be substantially the same for imaging the upper interior surface or the lower interior surface of the flow cell without moving one or more optical elements of the tube lens into or out of the optical path or without moving one or more optical elements of the tube lens along the optical path (e.g., optical axis) therebetween. Additional discussion of MTF is included below and in U.S. Provisional Application No. 62/962,723 filed Jan. 17, 2020, which is incorporated herein by reference in its entirety.

It will be understood by those of skill in the art that the disclosed imaging modules or systems may, in some instances, be stand-alone optical systems designed for imaging a sample or substrate surface. In some instances, they may comprise one or more processors or computers. In some instances, they may comprise one or more software packages that provide instrument control functionality and/or image processing functionality. In some instances, in addition to optical components such as light sources (e.g., solid-state lasers, dye lasers, diode lasers, arc lamps, tungsten-halogen lamps, etc.), lenses, prisms, mirrors, dichroic reflectors, beam splitters, optical filters, optical bandpass filters, light guides, optical fibers, apertures, and image sensors (e.g., complementary metal oxide semiconductor (CMOS) image sensors and cameras, charge-coupled device (CCD) image sensors and cameras, etc.), they may also include mechanical and/or optomechanical components, such as X-Y translation stages, X-Y-Z translation stages, piezoelectric focusing mechanisms, electro-optical phase plates, and the like. In some instances, they may function as modules, components, sub-assemblies, or sub-systems of larger systems designed for, e.g., genomics applications (e.g., genetic testing and/or nucleic acid sequencing applications). For example, in some instances, they may function

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as modules, components, sub-assemblies, or sub-systems of larger systems that further comprise light-tight and/or other environmental control housings, temperature control modules, flow cells and cartridges, fluidics control modules, fluid dispensing robotics, cartridge- and/or microplate-handling (pick-and-place) robotics, one or more processors or computers, one or more local and/or cloud-based software packages (e.g., instrument/system control software packages, image processing software packages, data analysis software packages), data storage modules, data communication modules (e.g., Bluetooth, WiFi, intranet, or internet communication hardware and associated software), display modules, etc., or any combination thereof. These additional components of larger systems, e.g., systems designed for genomics applications, will be discussed in more detail below.

FIGS. 2A and 2B illustrate a non-limiting example of an illumination and imaging module **100** for multi-channel fluorescence imaging. The illumination and imaging module **100** includes an objective lens **110**, an illumination source **115**, a plurality of detection channels **120**, and a first dichroic filter **130**, which may comprise a dichroic reflector or beam splitter. An autofocus system, which may include an autofocus laser **102**, for example, that projects a spot the size of which is monitored to determine when the imaging system is in-focus may be included in some designs. Some or all components of the illumination and imaging module **100** may be coupled to a baseplate **105**.

The illumination or light source **115** may include any suitable light source configured to produce light of at least a desired excitation wavelength (discussed in more detail below). The light source may be a broadband source that emits light within one or more excitation wavelength ranges (or bands). The light source may be a narrowband source that emits light within one or more narrower wavelength ranges. In some instances, the light source may produce a single isolated wavelength (or line) corresponding to the desired excitation wavelength, or multiple isolated wavelengths (or lines). In some instances, the lines may have some very narrow bandwidth. Example light sources that may be suitable for use in the illumination source **115** include, but are not limited to, an incandescent filament, xenon arc lamp, mercury-vapor lamp, a light-emitting diode, a laser source such as a laser diode or a solid-state laser, or other types of light sources. As discussed below, in some designs, the light source may comprise a polarized light source such as a linearly polarized light source. In some implementations, the orientation of the light source is such that s-polarized light is incident on one or more surfaces of one or more optical components such as the dichroic reflective surface of one or more dichroic filters.

The illumination source **115** may further include one or more additional optical components such as lenses, filters, optical fibers, or any other suitable transmissive or reflective optics as appropriate to output an excitation light beam having suitable characteristics toward a first dichroic filter **130**. For example, beam shaping optics may be included, for example, to receive light from a light emitter in the light source and produce a beam and/or provide a desired beam characteristic. Such optics may, for example, comprise a collimating lens configured to reduce the divergence of light and/or increase collimation and/or to collimate the light.

In some implementations, multiple light sources are included in the illumination and imaging module **100**. In some such implementations, different light sources may produce light having different spectral characteristics, for example, to excite different fluorescence dyes. In some implementations, light produced by the different light

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sources may be directed to coincide and form an aggregate excitation light beam. This composite excitation light beam may be composed of excitation light beams from each of the light sources. The composite excitation light beam will have more optical power than the individual beams that overlap to form the composite beam. For example, in some implementations that include two light sources that produce two excitation light beams, the composite excitation light beam formed from the two individual excitation light beams may have optical power that is the sum of the optical power of the individual beams. Similarly, in some implementations, three, four, five or more light sources may be included, and these light sources may each output excitation light beams that together form a composite beam that has an optical power that is the sum of the optical power of the individual beams.

In some implementations, the light source **115** outputs a sufficiently large amount of light to produce sufficiently strong fluorescence emission. Stronger fluorescence emission can increase the signal-to-noise ratio (SNR) and the contrast-to-noise ratio (CNR) of images acquired by the fluorescence imaging module. In some implementations, the output of the light source and/or an excitation light beam derived therefrom (including a composite excitation light beam) may range in power from about 0.5 W to about 5.0 W, or more (as will be discussed in more detail below).

Referring again to FIGS. 2A and 2B, the first dichroic filter **130** is disposed with respect to the light source to receive light therefrom. The first dichroic filter may comprise a dichroic mirror, dichroic reflector, dichroic beam splitter, or dichroic beam combiner configured to transmit light in a first spectral region (or wavelength range) and reflect light having a second spectral region (or wavelength range). The first spectral region may include one or more spectral bands, e.g., one or more spectral bands in the ultraviolet and blue wavelength ranges. Similarly, a second spectral region may include one or more spectral bands, e.g., one or more spectral bands extending from the green to red and infrared wavelengths. Other spectral regions or wavelength ranges are also possible.

In some implementations, the first dichroic filter may be configured to transmit light from the light source to a sample support structure such as to a microscope slide, a capillary, a flow cell, a microfluidic chip, or other substrate or support structure. The sample support structure supports and positions the sample, e.g., a composition comprising a fluorescently-labeled nucleic acid molecule or complement thereof, with respect to the illumination and imaging module **100**. Accordingly, a first optical path extends from the light source to the sample via the first dichroic filter. In various implementations, the sample support structure includes at least one surface on which the sample is disposed or to which the sample binds. In some instances, the sample may be disposed within or bound to different localized regions or sites on the at least one surface of the sample support structure.

In some instances, the support structure may include two surfaces located at different distances from objective lens **110** (i.e., at different positions or depths along the optical axis of objective lens **110**) on which the sample is disposed. As discussed below, for example, a flow cell may comprise a fluid channel formed at least in part by first and second (e.g., upper and lower) interior surfaces, and the sample may be disposed at localized sites on the first interior surface, the second interior surface, or both interior surfaces. The first and second surface may be separated by the region corresponding to the fluid channel through which a solution

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flows, and thus be at different distances or depth with respect to objective lens **110** of the illumination and imaging module **100**.

The objective lens **110** may be included in the first optical path between the first dichroic filter and the sample. This objective lens may be configured, for example, to have a focal length, working distance, and/or be positioned to focus light from the light source(s) onto the sample, e.g., onto a surface of the microscope slide, capillary, flow cell, microfluidic chip, or other substrate or support structure. Similarly, the objective lens **110** may be configured to have suitable focal length, working distance, and/or be positioned to collect light reflected, scattered, or emitted from the sample (e.g., fluorescence emission) and to form an image of the sample (e.g., a fluorescence image).

In some implementations, objective lens **110** may comprise a microscope objective such as an off-the-shelf objective. In some implementations, objective lens **110** may comprise a custom objective. An example of a custom objective lens and/or custom objective—tube lens combination is described below and in U.S. Provisional Application No. 62/962,723 filed on Jan. 17, 2020, which is incorporated herein by reference in its entirety. The objective lens **110** may be designed to reduce or minimize optical aberration at two locations such as two planes corresponding to two surfaces of a flow cell or other sample support structure. The objective lens **110** may be designed to reduce the optical aberration at the selected locations or planes, e.g., the first and second surfaces of a dual surface flow cell, relative to other locations or planes in the optical path. For example, the objective lens **110** may be designed to reduce the optical aberration at two depths or planes located at different distances from the objective lens as compared to the optical aberrations associated with other depths or planes at other distances from the objective. For example, in some instances, optical aberration may be less for imaging the first and second surfaces of a flow cell than that exhibited elsewhere in a region spanning from 1 to 10 mm from the front surface of the objective lens. Additionally, a custom objective lens **110** may in some instances be configured to compensate for optical aberration induced by transmission of fluorescence emission light through one or more portions of the sample support structure, such as a layer that includes one or more of the flow cell surfaces on which a sample is disposed, or a layer comprising a solution filling the fluid channel of a flow cell. These layers may comprise, e.g., glass, quartz, plastic, or other transparent material having a refractive index, and which may introduce optical aberration.

In some implementations, objective lens **110** may have a numerical aperture (NA) of 0.6 or more (as discussed in more detail below). Such a numerical aperture may provide for reduced depth of focus and/or depth of field, improved background discrimination, and increased imaging resolution.

In some implementations, objective lens **110** may have a numerical aperture (NA) of 0.6 or less (as discussed in more detail below). Such a numerical aperture may provide for increased depth of focus and/or depth of field. Such increased depth of focus and/or depth of field may increase the ability to image planes separated by a distance such as that that separates the first and second surfaces of a dual surface flow cell.

As discussed above, a flow cell may comprise, for example, first and second layers comprising first and second interior surfaces respectively that are separated by a fluid channel through which an analyte or reagent can flow. In

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some implementations, the objective lens **110** and/or illumination and imaging module **100** may be configured to provide a depth of field and/or depth of focus sufficiently large to image both the first and second interior surfaces of the flow cell, either sequentially by re-focusing the imaging module between imaging the first and second surfaces, or simultaneously by ensuring a sufficiently large depth of field and/or depth of focus, with comparable optical resolution. In some instances, the depth of field and/or depth of focus may be at least as large or larger than the distance separating the first and second surfaces of the flow cell to be imaged, such as the first and second interior surfaces of the flow cell. In some instances, the first and second surfaces, e.g., the first and second interior surfaces of a dual surface flow cell or other sample support structure, may be separated, for example, by a distance ranging from about 10 μm to about 700 μm , or more (as will be discussed in more detail below). In some instances, the depth of field and/or depth of focus may thus range from about 10 μm to about 700 μm , or more (as will be discussed in more detail below).

In some designs, compensation optics (e.g., an “optical compensator” or “compensator”) may be moved into or out of an optical path in the imaging module, for example, an optical path by which light collected by the objective lens **110** is delivered to an image sensor, to enable the imaging module to image the first and second surfaces of the dual surface flow cell. The imaging module may be configured, for example, to image the first surface when the compensation optics is included in the optical path between the objective lens and an image sensor or photodetector array configured to capture an image of the first surface. In such a design, the imaging module may be configured to image the second surface when the compensation optics is removed from or not included in the optical path between the objective lens **110** and the image sensor or photodetector array configured to capture an image of the second surface. The need for an optical compensator may be more pronounced when using an objective lens **110** with a high numerical aperture (NA) value, e.g., for numerical aperture values of at least 0.6, at least 0.65, at least 0.7, at least 0.75, at least 0.8, at least 0.85, at least 0.9, at least 0.95, at least 1.0, or higher. In some implementations, the optical compensation optics (e.g., an optical compensator or compensator) comprises a refractive optical element such as a lens, a plate of optically-transparent material such as glass, a plate of optically-transparent material such as glass, or in the case of polarized light beams, a quarter-wave plate or half-wave plate, etc. Other configurations may be employed to enable the first and second surfaces to be imaged at different times. For example, one or more lenses or optical elements may be configured to be translated in and out of, or along, an optical path between the objective lens **110** and the image sensor.

In certain designs, however, the objective lens **110** is configured to provide sufficiently large depth of focus and/or depth of field to enable the first and second surfaces to be imaged with comparable optical resolution without such compensation optics moving into and out of an optical path in the imaging module, such as an optical path between the objective lens and the image sensor or photodetector array. Similarly, in various designs, the objective lens **110** is configured to provide sufficiently large depth of focus and/or depth of field to enable the first and second surfaces to be imaged with comparable optical resolution without optics being moved, such as one or more lenses or other optical components being translated along an optical path in the imaging module, such as an optical path between the objec-

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tive lens and the image sensor or photodetector array. Examples of such objective lenses will be described in more detail below.

In some implementations, the objective lens (or microscope objective) **110** may be configured to have reduced magnification. The objective lens **110** may be configured, for example, such that the fluorescence imaging module has a magnification of from less than 2× to less than 10× (as will be discussed in more detail below). Such reduced magnification may alter design constraints such that other design parameters can be achieved. For example, the objective lens **110** may also be configured such that the fluorescence imaging module has a large field-of-view (FOV) ranging, for example, from about 1.0 mm to about 5.0 mm (e.g., in diameter, width, length, or longest dimension) as will be discussed in more detail below.

In some implementations, the objective lens **110** may be configured to provide the fluorescence imaging module with a field-of-view as indicated above such that the FOV has diffraction-limited performance, e.g., less than 0.15 waves of aberration over at least 60%, 70%, 80%, 90%, or 95% of the field, as will be discussed in more detail below.

In some implementations, the objective lens **110** may be configured to provide the fluorescence imaging module with a field-of-view as indicated above such that the FOV has diffraction-limited performance, e.g., a Strehl ratio of greater than 0.8 over at least 60%, 70%, 80%, 90%, or 95% of the field, as will be discussed in more detail below.

Referring again to FIGS. 2A and 2B, the first dichroic beam splitter or beam combiner is disposed in the first optical path between the light source and the sample so as to illuminate the sample with one or more excitation beams. This first dichroic beam splitter or combiner is also in one or more second optical path(s) from the sample to the different optical channels used to detect the fluorescence emission. Accordingly, the first dichroic filter **130** couples the first optical path of the excitation beam emitted by the illumination source **115** and second optical path of the emission light emitted by a sample specimen to the various optical channels where the light is directed to respective image sensors or photodetector arrays for capturing images of the sample.

In various implementations, the first dichroic filter **130**, e.g., first dichroic reflector or beam splitter or beam combiner, has a passband selected to transmit light from the illumination source **115** only within a specified wavelength band or possibly a plurality of wavelength bands that include the desired excitation wavelength or wavelengths. For example, the first dichroic beam splitter **130** includes a reflective surface comprising a dichroic reflector that has spectral transmissivity response that is, e.g., configured to transmit light having at least some of the wavelengths output by the light source that form part of the excitation beam. The spectral transmissivity response may be configured not to transmit (e.g., instead to reflect) light of one or more other wavelengths, for example, of one or more other fluorescence emission wavelengths. In some implementations, the spectral transmissivity response may also be configured not to transmit (e.g., instead to reflect) light of one or more other wavelengths output by the light source. Accordingly, the first dichroic filter **130** may be utilized to select which wavelength or wavelengths of light output by the light source reach the sample. Conversely, the dichroic reflector in the first dichroic beam splitter **130** has a spectral reflectivity response that reflects light having one or more wavelengths corresponding to the desired fluorescence emission from the sample and possible reflects light having one or more

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wavelengths output from the light source that is not intended to reach the sample. Accordingly, in some implementations, the dichroic reflector has a spectral transmissivity that includes one or more pass bands to transmit the light to be incident on the sample and one or more stop bands that reflects light outside the pass bands, for example, light at one or more emission wavelengths and possibly one or more wavelengths output by the light source that are not intended to reach the sample. Likewise, in some implementations the dichroic reflector has a spectral reflectivity that includes one or more spectral regions configured to reflect one or more emission wavelengths and possible one or more wavelengths output by the light source that are not intended to reach the sample and includes one or more regions that transmit light outside these reflection regions. The dichroic reflector included in the first dichroic filter **130** may comprise a reflective filter such as an interference filter (e.g., a quarter-wave stack) configured to provide the appropriate spectral transmission and reflection distributions. FIGS. 2A and 2B also show a dichroic filter **105**, which may comprise for example a dichroic beam splitter or beam combiner, that may be used to direct the autofocus laser **102** though the objective and to the sample support structure.

Although the imaging module **100** shown in FIGS. 2A and 2B and discussed above is configured such that the excitation beam is transmitted by the first dichroic filter **130** to the objective lens **110**, in some designs the illumination source **115** may be disposed with respect to the first dichroic filter **130** and/or the first dichroic filter is configured (e.g., oriented) such that the excitation beam is reflected by the first dichroic filter **130** to the objective lens **110**. Similarly, in some such designs, the first dichroic filter **130** is configured to transmit fluorescence emission from the sample and possibly transmit light having one or more wavelengths output from the light source that is not intended to reach the sample. As will be discussed below, a design where the fluorescence emission is transmitted instead of reflected may potentially reduce wavefront error in the detected emission and/or possibly have other advantages. In either case, in various implementations the first dichroic reflector **130** is disposed in the second optical path so as to receive fluorescence emission from the sample, at least some of which continues on to the detection channels **120**.

FIGS. 3A and 3B illustrate the optical paths within the multi-channel fluorescence imaging module of FIGS. 2A and 2B. In the example show in FIG. 2A and FIG. 3A, the detection channels **120** are disposed to receive fluorescence emission from a sample specimen that is transmitted by the objective lens **110** and reflected by the first dichroic filter **130**. As referred to above and described more below, in some designs the detection channels **120** may be disposed to receive the portion of the emission light that is transmitted, rather than reflected, by the first dichroic filter. In either case, the detection channels **120** may include optics for receiving at least a portion of the emission light. For example, the detection channels **120** may include one or more lenses, such as tube lenses, and may include one or more image sensors or detectors such as photodetector arrays (e.g., CCD or CMOS sensor arrays) for imaging or otherwise producing a signal based on the received light. The tube lenses may, for example, comprise one or more lens elements configured to form an image of the sample onto the sensor or photodetector array to capture an image thereof. Additional discussion of detection channels is included below and in U.S. Provisional Application No. 62/962,723, filed Jan. 17, 2020, which is incorporated herein by reference in its entirety. In some instances, improved optical resolution may be

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achieved using an image sensor having relatively high sensitivity, small pixels, and high pixel count, in conjunction with a suitable sampling scheme, which may include over-sampling or undersampling.

FIGS. 3A and 3B are ray tracing diagrams illustrating optical paths of the illumination and imaging module 100 of FIGS. 2A and 2B. FIG. 3A corresponds to a top view of the illumination and imaging module 100. FIG. 3B corresponds to a side view of the illumination and imaging module 100. The illumination and imaging module 100 illustrated in these figures includes four detection channels 120. However, it will be understood that the disclosed illumination and imaging modules may equally be implemented in systems including more or fewer than four detection channels 120. For example, the multi-channel systems disclosed herein may be implemented with as few as one detection channel 120, or as many as two detection channels 120, three detection channels 120, four detection channels 120, five detection channels 120, six detection channels 120, seven detection channels 120, eight detection channels 120, or more than eight detection channels 120, without departing from the spirit or scope of the present disclosure.

The non-limiting example of imaging module 100 illustrated in FIGS. 3A and 3B includes four detection channels 120, a first dichroic filter 130 that reflects a beam 150 of emission light, a second dichroic filter (e.g., a dichroic beam splitter) 135 that splits the beam 150 into a transmitted portion and a reflected portion, and two channel-specific dichroic filters (e.g., dichroic beam splitters) 140 that further split the transmitted and reflected portions of the beam 150 among individual detection channels 120. The dichroic reflecting surface in the dichroic beam splitters 135 and 140 for splitting the beam 150 among detection channels are shown disposed at 45 degrees relative to a central beam axis of the beam 150 or an optical axis of the imaging module. However, as discussed below, an angle smaller than 45 degrees may be employed and may offer advantages such as sharper transitions from pass band to stop band.

The different detection channels 120 includes imaging devices 124, which may include an image sensor or photodetector array (e.g., a CCD or CMOS detector array). The different detection channels 120 further include optics 126 such as lenses (e.g., one or more tube lenses each comprising one or more lens elements) disposed to focus the portion of the emission light entering the detection channel 120 at a focal plane coincident with a plane of the photodetector array 124. The optics 126 (e.g., a tube lens) combined with the objective lens 110 are configured to form an image of the sample onto the photodetector array 124 to capture an image of the sample, for example, an image of a surface on the flow cell or other sample support structure after the sample has bound to that surface. Accordingly, such an image of the sample may comprise a plurality of fluorescent emitting spots or regions across a spatial extent of the sample support structure where the sample is emitting fluorescence light. The objective lens 110 together with the optics 126 (e.g., tube lens) may provide a field of view (FOV) that includes a portion of the sample or the entire sample. Similarly, the photodetector array 124 of the different detection channels 120 may be configured to capture images of a full field of view (FOV) provided by the objective lens and the tube lens, or a portion thereof. In some implementations, the photodetector array 124 of some or all detection channels 120 can detect the emission light emitted by a sample disposed on the sample support structure, e.g., a surface of the flow cell, or a portion thereof and record electronic data representing an image thereof. In some implementations, the photodetector

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array 124 of some or all detection channels 120 can detect features in the emission light emitted by a specimen without capturing and/or storing an image of the sample disposed on the flow cell surface and/or of the full field of view (FOV) provided by the objective lens and optics 126 and/or 122 (e.g., elements of a tube lens). In some implementations, the FOV of the disclosed imaging modules (e.g., that provided by the combination of objective lens 110 and optics 126 and/or 122) may range, for example, between about 1 mm and 5 mm (e.g., in diameter, width, length, or longest dimension) as will be discussed below. The FOV may be selected, for example, to provide a balance between magnification and resolution of the imaging module and/or based on one or more characteristics of the image sensors and/or objective lenses. For example, a relatively smaller FOV may be provided in conjunction with a smaller and faster imaging sensor to achieve high throughput.

Referring again to FIGS. 3A and 3B, in some implementations, the optics 126 in the detection channel (e.g., the tube lens) may be configured to reduce optical aberration in images acquired using optics 126 in combination with objective lens 110. In some implementations comprising multiple detection channels for imaging at different emission wavelengths, the optics 126 (e.g., the tube lens) for different detection channels have different designs to reduce aberration for the respective emission wavelengths at which that particular channel is configured to image. In some implementations, the optics 126 (e.g., the tube lens) may be configured to reduce aberrations when imaging a specific surface (e.g., a plane, object plane, etc.) on the sample support structure comprising fluorescing sample sites disposed thereon as compared to other locations (e.g., other planes in object space). Similarly, in some implementations, the optics 126 (e.g., the tube lens) may be configured to reduce aberrations when imaging first and second surfaces (e.g., first and second planes, first and second object planes, etc.) on a dual surface sample support structure (e.g., a dual surface flow cell) having fluorescing sample sites disposed thereon as compared to other locations (e.g., other planes in object space). For example, the optics 126 in the detection channel (e.g., tube lens) may be designed to reduce the aberration at two depths or planes located at different distances from the objective lens as compared to the aberrations associated with other depths or planes at other distances from the objective. For example, optical aberration may be less for imaging the first and second surfaces than elsewhere in a region from about 1 to about 10 mm from the objective lens. Additionally, custom optic 126 in the detection channel (e.g., a tube lens) may in some embodiments be configured to compensate for aberration induced by transmission of emission light through one or more portions of the sample support structure such as a layer that includes one of the surfaces on which the sample is disposed as well as possibly a solution adjacent to and in contact with the surface on which the sample is disposed. The layer comprising one of the surfaces on which the sample is disposed may comprise, e.g., glass, quartz, plastic, or other transparent material having a refractive index, and which introduces optical aberration. Custom optic 126 in the detection channel (e.g., the tube lens), for example, may in some implementations be configured to compensate for optical aberration induced by a sample support structure, e.g., a coverslip or flow cell wall, or other sample support structure components, as well as possibly a solution adjacent to and in contact with the surface on which the sample is disposed.

In some implementations, the optics 126 in the detection channel (e.g., a tube lens) are configured to have reduced

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magnification. The optics **126** in the detection channel (e.g., a tube lens) may be configured, for example, such that the fluorescence imaging module has a magnification of less than, for example, 10×, as will be discussed further below. Such reduced magnification may alter design constraints such that other design parameters can be achieved. For example, the optics **126** (e.g., a tube lens) may also be configured such that the fluorescence imaging module has a large field-of-view (FOV), for example, of at least 1.0 mm or larger (e.g., in diameter, width, length, or longest dimension), as will be discussed further below.

In some implementations, the optics **126** (e.g., a tube lens) may be configured to provide the fluorescence imaging module with a field-of-view as indicated above such that the FOV has less than 0.15 waves of aberration over at least 60%, 70%, 80%, 90%, or 95% of the field, as will be discussed further below.

Referring again to FIGS. 3A and 3B, in various implementations, a sample is located at or near a focal position **112** of the objective lens **110**. As described above with reference to FIGS. 2A and 2B, a light source such as a laser source provides an excitation beam to the sample to induce fluorescence. At least a portion of fluorescence emission is collected by the objective lens **110** as emission light. The objective lens **110** transmits the emission light toward the first dichroic filter **130**, which reflects some or all of the emission light as the beam **150** incident upon the second dichroic filter **135** and to the different detection channels, each comprising optics **126** that form an image of the sample (e.g., a plurality of fluorescing sample sites on a surface of a sample support structure) onto a photodetector array **124**.

As discussed above, in some implementations, the sample support structure comprises a flow cell such as a dual surface flow cell having two surfaces (e.g., two interior surfaces, a first surface and a second surface, etc.) containing sample sites that emit fluorescent emission. These two surfaces may be separated by a distance from each other in the longitudinal (Z) direction along the direction of the central axis of the excitation beam and/or the optical axis of the objective lens. This separation may correspond, for example, to a flow channel within the flow cell. Analytes or reagents may be flowed through the flow channel and contact the first and second interior surfaces of the flow cell, which may thereby be contacted with a binding composition such that fluorescence emission is radiated from a plurality of sites on the first and second interior surfaces. The imaging optics (e.g., objective lens **110**) may be positioned at a suitable distance (e.g., a distance corresponding to the working distance) from the sample to form in-focus images of the sample on one or more detector arrays **124**. As discussed above, in various designs, the objective lens **110** (possibly in combination with the optics **126**) may have a depth of field and/or depth of focus that is at least as large as the longitudinal separation between the first and second surfaces. The objective lens **110** and the optics **126** (of each detection channel) can thus simultaneously form images of both the first and the second flow cell surfaces on the photodetector array **124**, and these images of the first and second surfaces are both in focus and have comparable optical resolution (or may be brought into focus with only minor refocusing of the objects to acquire images of the first and second surfaces that have comparable optical resolution). In various implementations, compensation optics need not be moved into or out of an optical path of the imaging module (e.g., into or out of the first and/or second optical paths) to form in-focus images of the first and second surfaces that are of comparable optical resolution. Similarly, in various implementations, one or more optical

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elements (e.g., lens elements) in the imaging module (e.g., the objective lens **110** or optics **126**) need not be moved, for example, in the longitudinal direction along the first and/or second optical paths to form in-focus images of the first surface in comparison to the location of said one or more optical elements when used to form in-focus images of the second surface. In some implementations, the imaging module includes an autofocus system configured to quickly and sequentially refocus the imaging module on the first and/or second surface such that the images have comparable optical resolution. In some implementations, objective lens **110** and/or optics **126** are configured such that both the first and second flow cell surfaces are in focus simultaneously with comparable optical resolution without moving an optical compensator into or out of the first and/or second optical path, and without moving one or more lens elements (e.g., objective lens **110** and/or optics **126** (such as a tube lens) longitudinally along the first and/or second optics path. In some implementations, images of the first and/or second surfaces, acquired either sequentially (e.g., with refocusing between surfaces) or simultaneously (e.g., without refocusing between surfaces) using the novel objective lens and/or tube lens designs disclosed herein, may be further processed using a suitable image processing algorithm to enhance the effective optical resolution of the images such that the images of the first and second surfaces have comparable optical resolution. In various implementations, the sample plane is sufficiently in focus to resolve sample sites on the first and/or second flow cell surfaces, the sample sites being closely spaced in lateral directions (e.g., in the X and Y directions).

As discussed above, the dichroic filters may comprise interference filters that selectively transmit and reflect light of different wavelengths based on the principle of thin-film interference, using layers of optical coatings having different refractive indices and particular thickness. Accordingly, the spectral response (e.g., transmission and/or reflection spectra) of the dichroic filters implemented within multi-channel fluorescence imaging modules may be at least partially dependent upon the angle of incidence, or range of angles of incidence, at which the light of the excitation and/or emission beams are incident upon the dichroic filters. Such effects may be especially significant with respect to the dichroic filters of the detection optical path (e.g., the dichroic filters **135** and **140** of FIGS. 3A and 3B).

FIG. 4 is a graph illustrating a relationship between dichroic filter performance and beam angle of incidence (AOI). Specifically, the graph of FIG. 4 illustrates the effect of angle of incidence on the transition width or spectral span of a dichroic filter, which corresponds to the range of wavelengths where the spectral response (e.g., transmission spectrum and/or reflection spectrum) transitions between the passband and stopband regions of a dichroic filter. Thus, a transmission edge (or reflection edge) having a relatively small spectral span (e.g., a small $\Delta\lambda$, value in the graph of FIG. 4) corresponds to a sharper transition between passband and stopband regions or the transmission and reflection regions (or conversely between reflection and transmission regions), while a transmission edge (or reflection edge) having a relatively large spectral span (e.g., a large $\Delta\lambda$, value in the graph of FIG. 4) corresponds to a less sharp transition between passband and stopband regions. In various implementations, sharper transitions between passband and stopband regions are generally desirable. Moreover, it may also be desirable to have increased consistency or a relatively consistent transition width across all or most of the field of view and/or beam area.

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Fluorescence imaging modules, in which the dichroic mirrors are disposed at 45 degrees relative to a central beam axis of the emission light or the optical axis of the optical paths (e.g., of the objective lens and/or tube lens), accordingly can have a transition width of roughly 50 nm for an example dichroic filter, as shown in FIG. 4. Because the emission light beam is not collimated and has some degree of divergence, fluorescence imaging modules may have a range of angles of incidence of approximately 5 degrees between opposing sides of the beam. Thus, as shown in FIG. 4, different portions of the beam of emission light may be incident upon a channel splitting dichroic filter at various angles of incidence between 40 degrees and 50 degrees. This range of relatively large angles of incidence corresponds to a range of transition widths between about 40 nm and about 62 nm. This range of relatively large angles of incidence thereby leads to an increase in transition width of the dichroic filter in the imaging module. Performance of multi-channel fluorescence imaging modules may thus be improved by providing smaller angles of incidence across the full beam, thereby making the transmission edge sharper and allowing better discrimination between different fluorescence emission bands.

FIG. 5 is a graph illustrating a relationship between beam footprint size (DBS) and beam angle of incidence (DBS angle) on a dichroic filter. In some instances, a smaller beam footprint may be desirable. For example, a small beam footprint allows smaller dichroic filters to be used to split a beam into different wavelength ranges. The use of smaller dichroic filters in turn reduces manufacturing costs and improves the ease of manufacturing suitably flat dichroic filters. As shown in FIG. 5, any angle of incidence greater than 0 degrees (i.e., perpendicular to the surface of the dichroic filter) results in an elliptical beam footprint having an area larger than the cross-sectional area of the beam. An angle of incidence of 45 degrees results in a large beam footprint on the dichroic reflector that is greater than 1.4 times the cross-sectional area of the beam when incident at zero degrees.

FIGS. 6A and 6B schematically illustrate a non-limiting example configuration of dichroic filters and detection channels in a multi-channel fluorescence imaging module where the dichroic mirrors are disposed at an angle of less than 45 degrees relative to a central beam axis of the emission light or the optical axis of the optical paths (e.g., of the objective lens and/or tube lens). FIG. 6A depicts an imaging module 500 including a plurality of detection channels 520a, 520b, 520c, 520d. FIG. 6B is a detailed view of the portion of the imaging module 500 within the circle 5B as shown in FIG. 6A. As will be described in greater detail, the configuration illustrated in FIGS. 6A and 6B includes a number of aspects that may result in significant improvements over conventional multi-channel fluorescence imaging module designs. In some instances, fluorescence imaging modules and systems of the present disclosure may, however, may be implemented with one or a subset of the features described with respect to FIGS. 6A and 6B without departing from the spirit or scope of the present disclosure.

The imaging module 500 depicted in FIG. 6A includes an objective lens 510 and four detection channels 520a, 520b, 520c, and 520d disposed to receive and/or image emission light transmitted by the objective lens 510. A first dichroic filter 530 is provided to couple the excitation and detection optical paths. In contrast to the design shown in FIGS. 2A and 2B, as well as in FIGS. 3A and 3B, the first dichroic filter 530 (e.g., a dichroic beam splitter or combiner) is configured to reflect light from the light source to the

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objective lens 510 and sample, and transmit fluorescence emission from the sample to the detection channels 520a, 520b, 520c, and 520d. A second dichroic filter 535 splits a beam of emission light among at least two detection channels 520a, 520b by transmitting a first portion 550a and reflecting a second portion 550b. Additional dichroic filters 540a, 540b are provided to further split the emission light. Dichroic filter 540a transmits at least a portion of the first portion 550a of the emission light and reflects a portion 550c to a third detection channel 520c. Dichroic filter 540b transmits at least a portion of the second portion 550b of the emission light and reflects a portion 550d to a fourth detection channel 520d. Although the imaging module 500 is depicted with four detection channels, in various embodiments the imaging module 500 may include more or fewer detection channels, with a correspondingly larger or smaller number of dichroic filters as appropriate to provide a portion of the emission light to each detection channel. For example, in some embodiments, the features of the imaging module 500 may be implemented with similar advantageous effects in a simplified imaging module including only two detection channels 520a, 520b, and omitting additional dichroic filters 540a, 540b. In some implementations, only one detection channel may be included. Alternatively, three or more detection channels may be employed.

The detection channels 520a, 520b, 520c, 520d illustrated in FIG. 6A may include some or all of the same or similar components to those of the detection channels 120 illustrated in FIGS. 2A-3B. For example, different detection channel 520a, 520b, 520c, 520d may include one or more image sensors or photodetectors arrays, and may include transmissive and/or reflective optics such as one or more lenses (e.g., tube lenses) that focus the light received by the detection channel onto its respective image sensor or photodetector array.

The objective lens 510 is disposed to receive emission light emitted by fluorescence from a specimen. In particular, the first dichroic filter 530 is disposed to receive the emission light collected and transmitted by the objective lens 510. As discussed above and shown in FIG. 6A, in some designs, an illumination source (e.g., the illumination source 115 of FIGS. 2A and 2B) such as a laser source or the like is disposed to provide an excitation beam which is incident on the first dichroic filter 530 such that the first dichroic filter 530 reflects the excitation beam into the same objective lens 510 that transmits the emission light, for example, in an epifluorescence configuration. In some other designs, the illumination source may be directed to the specimen by other optical components along a different optical path that does not include the same objective lens 510. In such configurations, the first dichroic filter 530 may be omitted.

Similarly, as discussed above and shown in FIG. 6A, the detection optics (e.g., including the detection channels 520a, 520b, 520c, 520d and any optical components such as dichroic filters 535, 540a, 540b along the optical path between the objective lens 510 and the detection channels 520a, 520b, 520c, 520d) may be disposed on the transmission path of the first dichroic filter 530, rather than on the reflected path of the first dichroic filter 530. In one example implementation, the objective lens 510 and detection optics are disposed such that the objective lens 510 transmits the beam 550 of emission light directly toward the second dichroic filter 535. The wavefront quality of the emission light may be degraded somewhat by the presence of the first dichroic filter 530 along the path of the beam 550 of emission light (e.g., by imparting some wavefront error to the beam 550). However, the wavefront error introduced by

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a beam transmitted through a dichroic reflector of a dichroic beam splitter is generally significantly smaller than the wavefront error of a beam reflected from the dichroic reflecting surface of a dichroic beam splitter (e.g., an order of magnitude smaller). Thus, the wavefront quality and subsequent imaging quality of the emission light in a multi-channel fluorescence imaging module may be substantially improved by placing the detection optics along the transmitted beam path of the first dichroic filter **530** rather than along the reflected beam path.

Still referring to FIG. 6A, within the detection optics of the imaging module **500**, dichroic filters **535**, **540a**, and **540b** are provided to split the beam **550** of emission light among the detection channels **520a**, **520b**, **520c**, **520d**. For example, the dichroic filters **535**, **540a**, and **540b** split the beam **550** on the basis of wavelength, such that a first wavelength or wavelength band of the emission light can be received by the first detection channel **520a**, a second wavelength or wavelength band of the emission light can be received by the second detection channel **520b**, a third wavelength or wavelength band of the emission light can be received by the third detection channel **520c**, and a fourth wavelength or wavelength band of the emission light can be received by the fourth detection channel **520d**. In some implementations, multiple separated wavelengths or wavelength bands can be received by the detection channel.

In contrast to the multi-channel fluorescence imaging module design shown in FIGS. 2A and 2B, as well as FIGS. 3A and 3B, the imaging module **500** has dichroic filters **535**, **540a**, and **540b** disposed at angles of incidence of less than 45 degrees with respect to the central beam axis of the incident beams. As shown in FIG. 6B, the different beams **550**, **550a**, **550b** have respective central beam axes **552**, **552a**, **552b**. In various implementations, the central beam axes **552**, **552a**, **552b** is at the center of a cross-section of the beam orthogonal to the propagation direction of the beam. These central beam axes **552**, **552a**, **552b** may correspond to the optical axis of the objective lens and/or the optics within the separate channels, for example, the optical axes of the respective tube lenses. Additional rays **554**, **554a**, **554b** of each beam **550**, **550a**, **550b** are illustrated in FIG. 6B to indicate the diameter of each beam **550**, **550a**, **550b**. Beam diameter may be defined, for example, as a full width at half maximum diameter, a $D4\sigma$ (i.e., 4 times σ , where σ is the standard deviation of the horizontal or vertical marginal distribution of the beam respectively) or second-moment width, or any other suitable definition of beam diameter.

The central beam axis **552** of the beam **550** of emission light may serve as a reference point for defining the angle of incidence of the beam **550** on the second dichroic filter **535**. Accordingly, the “angle of incidence” (AOI) of a beam **550** may be the angle between the central beam axis **552** of the incident beam **550** and a line N normal to the surface the beam is incident on, for example, the dichroic reflective surface. When the beam **550** of emission light is incident upon the dichroic reflective surface of the second dichroic filter **535** at an angle of incidence AOI, the second dichroic filter **535** transmits a first portion **550a** of the emission light (e.g., the portion having wavelengths within the passband region of the second dichroic filter **535**) and reflects a second portion **550b** of the emission light (e.g., the portion having wavelengths within the stopband region of the second dichroic filter **535**). The first portion **550a** and the second portion **550b** may each be similarly described in terms of a central beam axis **552a**, **552b**. As referred to above, the optical axis may alternatively or additionally be used.

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In the example configuration of FIGS. 6A and 6B, the second dichroic filter **535** is disposed such that the central beam axis **552** of the beam **550** is incident at an angle of incidence of 30 degrees. Similarly, the additional dichroic filters **540a**, **540b** are disposed such that the central beam axes **552a**, **552b** of the first and second portions **550a**, **550b** of the beam **550** are also incident at angles of incidence of 30 degrees. However, in various implementations these angles of incidence may be other angles smaller than 45 degrees. In some instances, for example, the angles of incidence may range between about 20 degrees and about 45 degrees, as will be discussed further below. Moreover, the angles of incidence on each of the dichroic filters **535**, **540a**, **540b** need not necessarily be the same. In some embodiments, some or all of the dichroic filters **535**, **540a**, **540b** may be disposed such that their incident beams **550**, **550a**, **550b** have different angles of incidence. As described above, the angle of incidence may be with respect to the optical axis of the optics within the imaging module, for example, the objective lens and/or the optics in the detection channels (e.g., the tube lenses) and the dichroic reflective surface in the respective dichroic beam splitter. The same ranges and values for the angle of incidence apply to the case when the optical axis is used to specify the AOI.

The beams **550**, **550a**, **550b** of emission light in a fluorescence imaging module system are typically diverging beams. As noted above, the beams of emission light can have a beam divergence large enough that regions of the beam within the beam diameter are incident upon the dichroic filters at angles of incidence that differ by up to 5 degrees or more relative to the angle of incidence of the central beam axis and/or optical axis of the optics. In some designs, the objective lens **510** may be configured, for example, to have an f-number or numerical aperture selected to produce a smaller beam diameter for a given field of view of the microscope. In one example, the f-number or numerical aperture of the objective lens **510** may be selected such that the full diameter of the beams **550**, **550a**, **550b** are incident upon dichroic filters **535**, **540a**, **540b** at angles of incidence within, for example, 1 degree, 1.5 degrees, 2 degrees, 2.5 degrees, 3 degrees, 3.5 degrees, 4 degrees, 4.5 degrees, or 5 degrees of the angle of incidence of the central beam axes **552**, **552a**, **552b**.

In some implementations, the focal length of the objective lens that is suitable for producing such a narrow beam diameter may be longer than those typically employed in fluorescence microscopes or imaging systems. For example, in some implementations, the focal length of the objective lens may range between 20 mm and 40 mm, as will be discussed further below. In one example, an objective lens **510** having a focal length of 36 mm may produce a beam **550** characterized by a divergence small enough that light across the full diameter of the beam **550** is incident upon the second dichroic filter **535** at angles within 2.5 degrees of the angle of incidence of the central beam axis.

FIG. 7 and FIG. 8 provide graphs illustrating improved dichroic filter performance due to aspects of the imaging module configuration of FIGS. 6A and 6B (or any of the imaging module configurations disclosed herein). The graph in FIG. 7 is similar to that of FIG. 4 and illustrates the effect of angle of incidence on the transition width (e.g., the spectral span of the transmission edge) of a dichroic filter. FIG. 7 shows an example where the orientation of a dichroic filter (e.g., dichroic filters **535**, **540a**, and **540b**) and the dichroic reflective surface therein is such that its incident beam has an angle of incidence of 30 degrees, rather than 45 degrees. FIG. 7 shows how this reduced angle of incidence

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significantly improves the sharpness and the uniformity of the transition width across the full beam diameter. For example, while an angle of incidence of 45 degrees at the central beam axis results in a range of transition widths between about 40 nm and about 62 nm, an angle of incidence of 30 degrees at the central beam axis results in a range of transition widths between about 16 nm and about 30 nm. In this example, the average transition width is reduced from about 51 nm to about 23 nm, indicating a sharper transition between passband and stopband. Moreover, the variation in transition widths across the beam diameter is reduced by nearly 40% from a 22 nm range to a 14 nm range, indicating a more uniform sharpness of the transition over the area of the beam.

FIG. 8 illustrates additional advantages that may be realized by selecting the appropriate f-number or numerical aperture for the objective lens to reduce beam divergence in any of the imaging module configurations disclosed herein. In some implementations, a longer focal length is used. In the example of FIG. 8, the objective lens 510 has a focal length of 36 mm, which with the appropriate numerical aperture (e.g., less than 5), reduces the range of angles of incidence within the beam 550 from 30 degrees \pm 5 degrees to 30 degrees \pm 2.5 degrees. With this design, the range of transition widths may be reduced to between about 19 nm and about 26 nm. When compared to the improved system of FIG. 7, although the average transition width is substantially the same (e.g., a spectral span of roughly 23 nm), the variation in transition widths across the beam diameter is further reduced to a 7 nm range, representing a reduction of nearly 70% relative to the range of transition widths illustrated in FIG. 4.

Referring again to FIG. 5, the reduction in angle of incidence from 45 degrees to 30 degrees at the central beam axis is further advantageous because it reduces the beam spot size on the dichroic filter. As shown in FIG. 5, an angle of incidence of 45 degrees results in a beam footprint on the dichroic filter having an area greater than 1.4 times the cross-sectional area of the beam. However, an angle of incidence of 30 degrees results in a beam footprint on the dichroic filter having an area only about 1.15 times the cross-sectional area of the beam. Thus, reducing the angle of incidence at the dichroic filters 535, 540a, 540b from 45 degrees to 30 degrees results in a reduction of about 18% in the area of the beam footprint on the dichroic filters 535, 540a, 540b. This reduction in beam footprint area allows smaller dichroic filters to be used.

Referring now jointly to FIGS. 9A-B, the reduction in angle of incidence from 45 degrees to 30 degrees may also provide improved performance with regard to surface deformation caused by the dichroic filters in any of the imaging module configurations disclosed herein, as indicated by improvements in the modulation transfer function. In general, the amount of surface deformation increases with larger area optical elements. If a larger area on the dichroic filter is employed, a larger amount of surface deformation is encountered, thereby introducing more wavefront error into the beam. FIG. 9A illustrates the effect of folding angle on image quality degradation induced by the addition of 1 wave of peak-to-valley (PV) spherical power to the last mirror. FIG. 9B illustrates the effect of folding angle on image quality degradation induced by the addition of 0.1 wave of PV spherical power to the last mirror. As shown in FIGS. 9A and 9B, the reduction in angle of incidence to 30 degrees significantly reduces the effect of surface deformation to achieve close to diffraction-limited performance of the detection optics.

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In some implementations of the disclosed imaging modules, the polarization state of the excitation beam may be utilized to further improve the performance of the multi-channel fluorescence imaging modules disclosed herein. Referring back to FIGS. 2A, 2B, and 6A, for example, some implementations of the multi-channel fluorescence imaging modules disclosed herein have an epifluorescence configuration in which a first dichroic filter 130 or 530 merges the optical paths of the excitation beam and the beam of emission light such that both the excitation and emission light are transmitted through the objective lens 110, 510. As discussed above, the illumination source 115 may include a light source such as a laser or other source which provides the light that forms the excitation beam. In some designs, the light source comprises a linearly polarized light source and the excitation beam may be linearly polarized. In some designs, polarization optics are included to polarize the light and/or rotate the polarization of the light. For example, a polarizer such as a linear polarizer may be included in an optical path of the excitation beam to polarize the excitation beam. Retarders such as half wave retarders or a plurality of quarter wave retarders or retarders having other amounts of retardance may be included to rotate the linear polarization in some designs.

The linearly polarized excitation beam, when it is incident upon any dichroic filter or other planar interface, may be p-polarized (e.g., having an electric field component parallel to the plane of incidence), s-polarized (e.g., having an electric field component normal to the plane of incidence), or may have a combination of p-polarization and s-polarization states within the beam. The p- or s-polarization state of the excitation beam may be selected and/or changed by selecting the orientation of the illumination source 115 and/or one or more components thereof with respect to the first dichroic filter 130, 530 and/or with respect to any other surfaces with which the excitation beam will interact. In some implementations where the light source output linearly polarized light, the light source can be configured to provide s-polarized light. For example, the light source may comprise an emitter such as a solid-state laser or a laser diode that may be rotated about its optical axis or the central axis of the beam to orient the linearly polarized light output therefrom. Alternatively, or in addition, retarders may be employed to rotate the linear polarization about the optical axis or the central axis of the beam. As discussed above, in some implementations, for example when the light source does not output polarized light, a polarizer disposed in the optical path of the excitation beam can polarize the excitation beam. In some designs, for example, a linear polarizer is disposed in the optical path of the excitation beam. This polarizer may be rotated to provide the proper orientation of the linear polarization to provide s-polarized light.

In some designs, the linear polarization is rotated about the optical axis or the central axis of the beam such that s-polarization is incident on the dichroic reflector of the dichroic beam splitter. When s-polarized light is incident on the dichroic reflector of the dichroic beam splitter the transition between the pass band and the stop band is sharper as opposed to when p-polarized light is incident on the dichroic reflector of the dichroic beam splitter.

As shown in FIGS. 10A and 10B, use of the p- or s-polarization state of the excitation beam may significantly affect the narrowband performance of any excitation filters such as the first dichroic filter 130, 530. FIG. 10A illustrates a transmission spectrum between 610 nm and 670 nm for an example bandpass dichroic filter at angles of incidence of 40 degrees and 45 degrees, where the incident beam is linearly

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polarized and is p-polarized with respect to the plane of the dichroic filter. As shown in FIG. 10B, changing the orientation of the light source with respect to the dichroic filter, such that the incident beam is s-polarized with respect to the plane of the dichroic filter, results in a substantially sharper edge between the passband and the stopband of the dichroic filter. Thus, the illumination and imaging modules 100, 500 disclosed herein may advantageously have an illumination source 115 oriented relative to the first dichroic filter 130, 530 such that the excitation beam is s-polarized with respect to the plane of the first dichroic filter 130, 530. As discussed above, in some implementation, a polarizer such as a linear polarizer may be used to polarize the excitation beam. This polarizer may be rotated to provide an orientation of the linearly polarized light corresponding to s-polarized light. Also as discussed above, in some implementations, other approaches to rotating the linearly polarized light may be used. For example, optical retarders such as half wave retarders or multiple quarter wave retarders may be used to rotate the polarization direction. Other arrangements are also possible.

As discussed elsewhere herein, reducing the numerical aperture (NA) of the fluorescence imaging module and/or of the objective lens may increase the depth of field to enable the comparable imaging of the two surfaces. FIGS. 11A-16B, show how the MTF is more similar at first and second surfaces separated by 1 mm of glass for lower numerical apertures than for larger numerical apertures.

FIGS. 11A and 11B show the MTF at first (FIG. 11A) and second (FIG. 11B) surfaces for an NA of 0.3.

FIGS. 12A and 12B show the MTF at first (FIG. 12A) and second (FIG. 12B) surfaces for an NA of 0.4.

FIGS. 13A and 13B show the MTF at first (FIG. 13A) and second (FIG. 13B) surfaces for an NA of 0.5.

FIGS. 14A and 14B show the MTF at first (FIG. 14A) and second (FIG. 14B) surfaces for an NA of 0.6.

FIGS. 15A and 15B show the MTF at first (FIG. 15A) and second (FIG. 15B) surfaces for an NA of 0.7.

FIGS. 16A and 16B show the MTF at first (FIG. 16A) and second (FIG. 16B) surfaces for an NA of 0.8. The first and second surfaces in each of these figures correspond to, e.g., the top and bottom surfaces of a flow cell.

FIGS. 17A-B provide plots of the calculated Strehl ratio (i.e., the ratio of peak light intensity focused or collected by the optical system versus that focused or collected by an ideal optical system and point light source) for imaging a second flow cell surface through a first flow cell surface. FIG. 17A shows a plot of the Strehl ratios for imaging a second flow cell surface through a first flow cell surface as a function of the thickness of the intervening fluid layer (fluid channel height) for different objective lens and/or optical system numerical apertures. As shown, the Strehl ratio decreases with increasing separation between the first and second surfaces. One of the surfaces would thus have deteriorated image quality with increasing separation between the two surfaces. The decrease in second surface imaging performance with increased separation distance between the two surfaces is reduced for imaging systems having smaller numerical apertures as compared to those having larger numerical apertures. FIG. 17B shows a plot of the Strehl ratio as a function of numerical aperture for imaging a second flow cell surface through a first flow cell surface and an intervening layer of water having a thickness of 0.1 mm. The loss of imaging performance at higher numerical apertures may be attributed to the increased optical aberration induced by the fluid for the second surface imaging. With increasing NA, the increased optical aberration

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introduced by the fluid for the second surface imaging degrades the image quality significantly. In general, however, reducing the numeral aperture of the optical system reduces the achievable resolution. This loss of image quality can be at least partially offset by providing an increased sample plane (or object plane) contrast-to-noise ratio, for example, by using chemistries for nucleic acid sequencing applications that enhance the fluorescence emission for labeled nucleic acid clusters and/or that reduce background fluorescence emission. In some instances, for example, sample support structures comprising hydrophilic substrate materials and/or hydrophilic coatings may be employed. In some cases, such hydrophilic substrates and/or hydrophilic coatings may reduce background noise. Additional discussion of sample support structures, hydrophilic surfaces and coatings, and methods for enhancing contrast-to-noise ratios, e.g., for nucleic acid sequencing applications, can be found below.

In some implementations, any one or more of the fluorescence imaging system, the illumination and imaging module 100, the imaging optics (e.g., optics 126), the objective lens, and/or the tube lens is configured to have reduced magnification, such as a magnification of less than 10 \times , as will be discussed further below. Such reduced magnification may adjust design constraints such that other design parameters can be achieved. For example, any one or more of the fluorescence microscope, illumination and imaging module 100, the imaging optics (e.g., optics 126), the objective lens or the tube lens may also be configured such that the fluorescence imaging module has a large field-of-view (FOV), for example, a field-of-view of at least 3.0 mm or larger (e.g., in diameter, width, height, or longest dimension), as will be discussed further below. Any one or more of the fluorescence imaging system, the illumination and imaging module 100, the imaging optics (e.g., optics 126), the objective lens and/or the tube lens may be configured to provide the fluorescence microscope with such a field-of-view such that the FOV has less than, e.g., 0.1 waves of aberration over at least 80% of field. Similarly, any one or more of the fluorescence imaging system, illumination and imaging module 100, the imaging optics (e.g., optics 126), the objective lens and/or the tube lens may be configured such that the fluorescence imaging module has such a FOV and is diffraction limited or is diffraction limited over such an FOV.

As discussed above, in various implementations, a large field-of-view (FOV) is provided by the disclosed optical systems. In some implementations, obtaining an increased FOV is facilitated in part by the use of larger image sensors or photodetector arrays. The photodetector array, for example, may have an active area with a diagonal of at least 15 mm or larger, as will be discussed further below. As discussed above, in some implementations the disclosed optical imaging systems provide a reduced magnification, for example, of less than 10 \times which may facilitate large FOV designs. Despite the reduced magnification, the optical resolution of the imaging module may still be sufficient as detector arrays having small pixel size or pitch may be used. The pixel size and/or pitch may, for example, be about 5 μ m or less, as will be discussed in more detail below. In some implementations, the pixel size is smaller than twice the optical resolution provided by the optical imaging system (e.g., objective and tube lens) to satisfy the Nyquist theorem. Accordingly, the pixel dimension and/or pitch for the image sensor(s) may be such that a spatial sampling frequency for the imaging module is at least twice an optical resolution of the imaging module. For example, the spatial sampling

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frequency for the photodetector array may be is at least 2 times, at least 2.5 times, at least 3 times, at least 4 times, or at least 5 times the optical resolution of the fluorescence imaging module (e.g., the illumination and imaging module, the objective and tube lens, the object lens and optics **126** in the detection channel, the imaging optics between the sample support structure or stage configured to support the sample support stage and the photodetector array) or any spatial sampling frequency in a range between any of these values.

Although a wide range of features are discussed herein with respect to fluorescence imaging modules, any of the features and designs describe herein may be applied to other types of optical imaging systems including without limitation bright-field and dark-field imaging, and may apply to luminescence or phosphorescence imaging.

Dual Wavelength Excitation/Four Channel Imaging System:

FIG. **18** illustrates a dual excitation wavelength/four channel imaging system for dual-side imaging applications that includes an objective and tube lens combination that is scanned in a direction perpendicular to the optical axis to provide for large area imaging, e.g., by tiling several images to create a composite image having a total field-of-view (FOV) that is much larger than that for each individual image. The system comprises two excitation light sources, e.g., lasers or laser diodes, operating at different wavelengths and an autofocus laser. The two excitation light beams and autofocus laser beam are combined using a series of mirrors and/or dichroic reflectors and delivered to an upper or lower interior surface of the flow cell through the objective. Fluorescence that is emitted by labeled oligonucleotides (or other biomolecules) tethered to one of the flow cell surfaces is collected by the objective, transmitted through the tube lens, and directed to one of four imaging sensors according to the wavelength of the emitted light by a series of intermediate dichroic reflectors. Autofocus laser light that has been reflected from the flow cell surface is collected by the objective, transmitted through the tube lens, and directed to an autofocus sensor by a series of intermediate dichroic reflectors. The system allows accurate focus to be maintained (e.g., by adjusting the relative distance between the flow cell surface and the objective using a precision linear actuator, translation stage, or microscope turret-mounted focus adjustment mechanism, to reduce or minimize the reflected light spot size on the autofocus image sensor) while the objective/tube lens combination is scanned in a direction perpendicular to the optical axis of the objective. Dual wavelength excitation used in combination with four channel (i.e. four wavelength) imaging capability provides for high-throughput imaging of the upper (near) and lower (far) interior surfaces of the flow cell.

Multiplexed Optical Read-Heads:

In some instances, miniaturized versions of any of the imaging modules described herein may be assembled to create a multiplexed read-head that may be translated in one or more directions horizontally relative to a sample surface, e.g., an interior surface of a flow cell, to image several sections of the surface simultaneously. A non-limiting example of a multiplexed read-head has recently been described in U.S. Published Patent Application No. 2020/0139375 A1.

In some instances, for example, a miniaturized imaging module may comprise a “microfluorometer” comprising an illumination or excitation light source such as an LED or laser diode (or the tip of an optical fiber connected to an external light source), one or more lenses for collimating or

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focusing the illumination or excitation light, one or more dichroic reflectors, one or more optical filters, one or more mirrors, beam-splitters, prisms, apertures, etc., one or more objectives, one or more custom tube lenses for enabling dual surface imaging with minimal focus adjustment as described elsewhere herein, one or more image sensors, or any combination thereof, as described elsewhere herein. In some instances, a miniaturized imaging module (e.g., a “microfluorometer”) may further comprise an autofocus mechanism, a microprocessor, power and data transfer connectors, a light-tight housing, etc. The resulting miniaturized imaging module may thus comprise an integrated imaging package or unit having a small form factor. In some instances, the shortest dimension (e.g., width or diameter) of the miniaturized imaging module may be less than 5 cm, less than 4.5 cm, less than 4 cm, less than 3.5 cm, less than 3 cm, less than 2.5 cm, less than 2 cm, less than 1.8 cm, less than 1.6 cm, less than 1.4 cm, less than 1.2 cm, less than 1 cm, less than 0.8 cm, or less than 0.6 cm. In some instances, the longest dimension (e.g., height or length) of the miniaturized imaging module may be less than 16 cm, less than 14 cm, less than 12 cm, less than 10 cm, less than 9 cm, less than 8 cm, less than 7 cm, less than 5 cm, less than 5 cm, less than 4.5 cm, less than 4 cm, less than 3.5 cm, less than 3 cm, less than 2.5 cm, less than 2 cm, less than 1.8 cm, less than 1.6 cm, less than 1.4 cm, less than 1.2 cm, or less than 1 cm. In some instances, one or more individual miniaturized imaging modules within the multiplexed read-head may comprise an autofocus mechanism.

In some instances, multiplexed read-heads as described herein may comprise an assembly of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more than 12 miniaturized imaging modules or microfluorometers held in fixed position relative to each other. In some instances, the optical design specifications and performance properties of the individual miniaturized imaging modules or microfluorometers, e.g. for numerical aperture, field-of-view, depth-of-field, image resolution, etc., may be the same as described elsewhere herein for other versions of the disclosed imaging modules. In some instances, the plurality of individual miniaturized imaging modules may be arranged in a linear arrangement comprising one, two, three, four, or more than four rows and/or columns. In some instances, the plurality of individual miniaturized imaging modules may be arranged in, e.g., a hexagonal close pack arrangement. In some instances, the plurality of individual miniaturized imaging modules may be arranged in a circular or spiral arrangement, a randomly distributed arrangement, or in any other arrangement known to those of skill in the art.

FIGS. **43A-B** provide non-limiting schematic illustrations of a multiplexed read-head as disclosed herein. FIG. **43A** shows a side view of a multiplexed read-head in which two rows of individual microfluorometers (seen from the end on) having common optical design specifications, e.g., numerical aperture, field-of-view, working distance, etc., are configured to image a common surface, e.g., a first interior surface of a flow cell. FIG. **43B** shows a top view of the same multiplexed read-head illustrating the overlapping imaging paths acquired by individual microfluorometers of the multiplexed read-head as the read-head is translated relative to the flow cell (or vice versa). In some instances, the individual fields-of-view for the individual microfluorometers may overlap, as indicated in FIG. **43B**. In some instances, they may not overlap. In some instances, the multiplexed-read head may be designed such that it aligns with and images predetermined features, e.g., individual fluid channels, within a flow cell.

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FIGS. 44A-B provide non-limiting schematic illustrations of a multiplexed read-head where a first subset of the plurality of individual miniaturized imaging modules is configured to image a first sample plane, e.g., a first interior surface of a flow cell, and a second subset of the plurality is configured to simultaneously image a second sample plane, e.g., a second interior surface of a flow cell. FIG. 44A shows a side view of the multiplexed read-head in which the first subset of individual microfluorimeters is configured to image, e.g., the first or upper interior surface of a flow cell, and the second subset is configured to image a second surface, e.g., the second or lower interior surface of a flow cell. FIG. 44B shows a top view of the multiplexed read-head of FIG. 44A illustrating the imaging paths acquired by individual microfluorimeters of the multiplexed read-head. Again, in some instances, the individual fields-of-view for the individual microfluorimeters in a given subset may overlap. In some instances, they may not overlap. In some instances, the multiplexed-read head may be designed such that the individual miniaturized imaging modules of the first and second subsets align with and image predetermined features, e.g., individual fluid channels, within a flow cell.

Improved or Optimized Objective and/or Tube Lens for Use with Thicker Coverslips:

Existing design practice includes the design of objective lenses and/or use of commonly available off-the-shelf microscope objectives to optimize image quality when images are acquired through thin (e.g., <200 μm thick) microscope coverslips. When used to image on both sides of a fluidic channel or flow cell, the extra height of the gap between the two surfaces (i.e., the height of the fluid channel; typically, about 50 μm to 200 μm) introduces optical aberration in images captured for the non-optimal side of the fluidic channel, thereby causing lower optical resolution. This is primarily because the additional gap height is significant compared to the optimal coverslip thickness (typical fluid channel or gap heights of 50-200 μm vs. coverslip thicknesses of <200 μm). Another common design practice is to utilize an additional “compensator” lens in the optical path when imaging is to be performed on the non-optimal side of the fluid channel or flow cell. This “compensator” lens and the mechanism required to move it in or out of the optical path so that either side of the flow cell may be imaged further increases system complexity and imaging system down time, and potentially degrades image quality due to vibration, etc.

In the present disclosure, the imaging system is designed for compatibility with flow cell consumables that comprise a thicker coverslip or flow cell wall (thickness $\geq 700 \mu\text{m}$). The objective lens design may be improved or optimized for a coverslip that is equal to the true cover slip thickness plus half of the effective gap thickness (e.g., $700 \mu\text{m} + \frac{1}{2} \times \text{fluid channel (gap) height}$). This design significantly reduces the effect of gap height on image quality for the two surfaces of the fluid channel and balances the optical quality for images of the two surfaces, as the gap height is small relative to the total coverslip thickness and thus its impact on optical quality is reduced.

Additional advantages of using a thicker coverslip include improved control of thickness tolerance error during manufacturing, and a reduced likelihood that the coverslip undergoes deformation due to thermal and mounting-induced stress. Coverslip thickness error and deformation adversely impact imaging quality for both the top surface and the bottom surface of a flow cell.

To further improve the dual surface imaging quality for sequencing applications, our optical system design places a

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strong emphasis on improving or optimizing MTF (e.g., through improving or optimizing the objective lens and/or tube lens design) in the mid- to high-spatial frequency range that is most suitable for imaging and resolving small spots or clusters.

Improved or Optimized Tube Lens Design for Use in Combination with Commercially Available, Off-the-Shelf Objectives:

For low-cost sequencer design, the use of a commercially available, off-the-shelf objective lens may be preferred due to its relatively low price. However, as noted above, low-cost, off-the-shelf objectives are mostly optimized for use with thin coverslips of about 170 μm in thickness. In some instances, the disclosed optical systems may utilize a tube lens design that compensates for a thicker flow cell coverslip while enabling high image quality for both interior surfaces of a flow cell in dual-surface imaging applications. In some instances, the tube lens designs disclosed herein enable high quality imaging for both interior surfaces of a flow cell without moving an optical compensator into or out of the optical path between the flow cell and an image sensor, without moving one or more optical elements or components of the tube lens along the optical path, and without moving one or more optical elements or components of the tube lens into or out of the optical path.

FIG. 19 provides an optical ray tracing diagram for a low light objective lens design that has been improved or optimized for imaging a surface on the opposite side of a 0.17 mm thick coverslip. The plot of modulation transfer function for this objective, shown in FIG. 20, indicates near-diffraction limited imaging performance when used with the designed-for 0.17 mm thick coverslip.

FIG. 21 provides a plot of the modulation transfer function for the same objective lens illustrated in FIG. 19 as a function of spatial frequency when used to image a surface on the opposite side of a 0.3 mm thick coverslip. The relatively minor deviations of MTF value over the spatial frequency range of about 100 to about 800 lines/mm (or cycles/mm) indicates that the image quality obtained even when using a 0.3 mm thick coverslip is still reasonable.

FIG. 22 provides a plot of the modulation transfer function for the same objective lens illustrated in FIG. 19 as a function of spatial frequency when used to image a surface that is separated from that on the opposite side of a 0.3 mm thick coverslip by a 0.1 mm thick layer of aqueous fluid (i.e., under the kind of conditions encountered for dual-side imaging of a flow cell when imaging the far surface). As can be seen in the plot of FIG. 22, imaging performance is degraded, as indicated by the deviations of the MTF curves from those for the an ideal, diffraction-limited case over the spatial frequency range of about 50 lp/mm to about 900 lp/mm.

FIG. 23 and FIG. 24 provide plots of the modulation transfer function as a function of spatial frequency for the upper (or near) interior surface (FIG. 23) and lower (or far) interior surface (FIG. 24) of a flow cell when imaged using the objective lens illustrated in FIG. 19 through a 1.0 mm thick coverslip, and when the upper and lower interior surfaces are separated by a 0.1 mm thick layer of aqueous fluid. As can be seen, imaging performance is significantly degraded for both surfaces.

FIG. 25 provides a ray tracing diagram for a tube lens design which, if used in conjunction with the objective lens illustrated in FIG. 19, provides for improved dual-side imaging through a 1 mm thick coverslip. The optical design 700 comprising a compound objective (lens elements 702, 703, 704, 705, 706, 707, 708, 709, and 710) and a tube lens

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(lens elements **711**, **712**, **713**, and **714**) is improved or optimized for use with flow cells comprising a thick coverslip (or wall), e.g., greater than 700 μm thick, and a fluid channel thickness of at least 50 μm , and transfers the image of an interior surface from the flow cell **701** to the image sensor **715** with dramatically improved optical image quality and higher CNR.

In some instances, the tube lens (or tube lens assembly) may comprise at least two optical lens elements, at least three optical lens elements, at least four optical lens elements, at least five optical lens elements, at least six optical lens elements, at least seven optical lens elements, at least eight optical lens elements, at least nine optical lens elements, at least ten optical lens elements, or more, where the number of optical lens elements, the surface geometry of each element, and the order in which they are placed in the assembly is improved or optimized to correct for optical aberrations induced by the thick wall of the flow cell, and in some instances, allows one to use a commercially-available, off-the-shelf objective while still maintaining high-quality, dual-side imaging capability.

In some instances, as illustrated in FIG. **25**, the tube lens assembly may comprise, in order, a first asymmetric convex-convex lens **711**, a second convex-plano lens **712**, a third asymmetric concave-concave lens **713**, and a fourth asymmetric convex-concave lens **714**.

FIG. **26** and FIG. **27** provide plots of the modulation transfer function as a function of spatial frequency for the upper (or near) interior surface (FIG. **26**) and lower (or far) interior surface (FIG. **27**) of a flow cell when imaged using the objective lens (corrected for a 0.17 mm coverslip) and tube lens combination illustrated in FIG. **25** through a 1.0 mm thick coverslip, and when the upper and lower interior surfaces are separated by a 0.1 mm thick layer of aqueous fluid. As can be seen, the imaging performance achieved is nearly that expected for a diffraction-limited optical design.

FIG. **28** provides ray tracing diagrams for tube lens design (left) of the present disclosure that has been improved or optimized to provide high-quality, dual-side imaging performance. Because the tube lens is no longer infinity-corrected, an appropriately designed null lens (right) may be used in combination with the tube lens to compensate for the non-infinity-corrected tube lens for manufacturing and testing purposes.

Imaging Channel-Specific Tube Lens Adaptation or Optimization:

In imaging system design, it is possible to improve or optimize both the objective lens and the tube lens in the same wavelength region for all imaging channels. Typically, the same objective lens is shared by all imaging channels (see, for example, FIG. **18**), and each imaging channel either uses the same tube lens or has a tube lens that shares the same design.

In some instances, the imaging systems disclosed herein may further comprise a tube lens for each imaging channel where the tube lens has been independently improved or optimized for the specific imaging channel to improve image quality, e.g., to reduce or minimize distortion and field curvature, and improve depth-of-field (DOF) performance for each channel. Because the wavelength range (or band-pass) for each specific imaging channel is much narrower than the combined wavelength range for all channels, the wavelength- or channel-specific adaptation or optimization of the tube lens used in the disclosed systems results in significant improvements in imaging quality and performance. This channel-specific adaptation or optimization

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results in improved image quality for both the top and bottom surfaces of the flow cell in dual-side imaging applications.

Dual-Side Imaging w/o Fluid Present in Flow Cell:

For optimal imaging performance of both top and bottom interior surfaces of a flow cell, a motion-actuated compensator is typically required to correct for optical aberrations induced by the fluid in the flow cell (typically comprising a fluid layer thickness of about 50-200 μm). In some instances of the disclosed optical system designs, the top interior surface of the flow cell may be imaged with fluid present in the flow cell. Once the sequencing chemistry cycle has been completed, the fluid may be extracted from the flow cell for imaging of the bottom interior surface. Thus, in some instances, even without the use of a compensator, the image quality for the bottom surface is maintained.

Compensation for Optical Aberration and/or Vibration Using Electro-Optical Phase Plates:

In some instances, dual-surface image quality may be improved without requiring the removal of the fluid from the flow cell by using an electro-optical phase plate (or other corrective lens) in combination with the objective to cancel the optical aberrations induced by the presence of the fluid. In some instances, the use of an electro-optical phase plate (or lens) may be used to remove the effects of vibration arising from the mechanical motion of a motion-actuated compensator and may provide faster image acquisition times and sequencing cycle times for genomic sequencing applications.

Improved Contrast-to-Noise Ratio (CNR), Field-of-View (FOV), Spectral Separation, and Timing Design to Increase or Maximize Information Transfer and Throughput:

Another way to increase or maximize information transfer in imaging systems designed for genomics applications is to increase the size of the field-of-view (FOV) and reduce the time required to image a specific FOV. With typical large NA optical imaging systems, it may be common to acquire images for fields-of-view that are on the order of 1 mm^2 in area, where in the presently disclosed imaging system designs large FOV objectives with long working distances are specified to enable imaging of areas of 2 mm^2 or larger.

In some cases, the disclosed imaging systems are designed for use in combination with proprietary low-binding substrate surfaces and DNA amplification processes that reduce fluorescence background arising from a variety of confounding signals including, but are not limited to, nonspecific adsorption of fluorescent dyes to substrate surfaces, nonspecific nucleic acid amplification products (e.g., nucleic acid amplification products that arise the substrate surface in areas between the spots or features corresponding to clonally-amplified clusters of nucleic acid molecules (i.e., specifically amplified colonies), nonspecific nucleic acid amplification products that may arise within the amplified colonies, phased and pre-phased nucleic acid strands, etc. The use of low-binding substrate surfaces and DNA amplification processes that reduce fluorescence background in combination with the disclosed optical imaging systems may significantly cut down on the time required to image each FOV.

The presently disclosed system designs may further reduce the required imaging time through imaging sequence improvement or optimization where multiple channels of fluorescence images are acquired simultaneously or with overlapping timing, and where spectral separation of the fluorescence signals is designed to reduce cross-talks between fluorescence detection channels and between the excitation light and the fluorescence signal(s).

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The presently disclosed system designs may further reduce the required imaging time through improvement or optimization of scanning motion sequence. In the typical approach, an X-Y translation stage is used to move the target FOV into position underneath the objective, an autofocus step is performed where optimal focal position is determined and the objective is moved in the Z direction to the determined focal position, and an image is acquired. A sequence of fluorescence images is acquired by cycling through a series of target FOV positions. From an information transfer duty cycle perspective, information is only transferred during the fluorescence image acquisition portion of the cycle. In the presently disclosed imaging system designs, a single-step motion in which all axes (X-Y-Z) are repositioned simultaneously is performed, and the autofocus step is used to check focal position error. The additional Z motion is only commanded if the focal position error (i.e., the difference between the focal plane position and the sample plane position) exceeds a certain limit (e.g., a specified error threshold). Coupled with high speed X-Y motion, this approach increases the duty cycle of the system, and thus increases the imaging throughput per unit time.

Furthermore, by matching the optical collection efficiency, modulation transfer function, and image sensor performance characteristics of the design with the fluorescence photon flux expected for the input excitation photon flux, dye efficiency (related to dye extinction coefficient and fluorescence quantum yield), while accounting for background signal and system noise characteristics, the time required to acquire high quality (high contrast-to-noise ratio (CNR) images) may be reduced or minimized.

The combination of efficient image acquisition and improved or optimized translation stage step and settle times leads to fast imaging times (i.e., the overall time required per field-of-view) and higher throughput imaging system performance.

Along with the large FOV and fast image acquisition duty cycle, the disclosed designs may comprise also specifying image plane flatness, chromatic focus performance between fluorescence detection channels, sensor flatness, image distortion, and focus quality specifications.

Chromatic focus performance is further improved by individually aligning the image sensors for different fluorescence detection channels such that the best focal plane for each detection channel overlaps. The design goal is to ensure that images across more than 90 percent of the field-of-view are acquired within ± 100 nm (or less) relative to the best focal plane for each channel, thus increasing or maximizing the transfer of individual spot intensity signals. In some instances, the disclosed designs further ensure that images across 99 percent of the field-of-view are acquired within ± 150 nm (or less) relative to the best focal plane for each channel, and that images across more the entire field-of-view are acquired within ± 200 nm (or less) relative to the best focal plane for each imaging channel.

Illumination Optical Path Design:

Another factor for improving signal-to-noise ratio (SNR), contrast-to-noise ratio (CNR), and/or increasing throughput is to increase illumination power density to the sample. In some instances, the disclosed imaging systems may comprise an illumination path design that utilizes a high-power laser or laser diode coupled with a liquid light guide. The liquid light guide removes optical speckle that is intrinsic to coherent light sources such as lasers and laser diodes. Furthermore, the coupling optics are designed in such a way as to underfill the entrance aperture of the liquid light guide. The underfilling of the liquid light guide entrance aperture

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reduces the effective numerical aperture of the illumination beam entering the objective lens, and thus improves light delivery efficiency through the objective onto the sample plane. With this design innovation, one can achieve illumination power densities up to $3\times$ that for conventional designs over a large field-of-view (FOV).

By utilizing the angle-dependent discrimination of s- and p-polarization, in some instances, the illumination beam polarization may be orientated to reduce the amount of back-scattered and back-reflected illumination light that reaches the imaging sensors.

Structured Illumination Systems:

In some instances, the disclosed imaging modules and systems may comprise a structured illumination optical design to increase the effective spatial resolution of the imaging system and thus enable the use of higher surface densities of clonally-amplified target nucleic acid sequences (clusters) on flow cell surfaces for improved sequencing throughput. Structured illumination microscopy (SIM) utilizes spatially structured (i.e., periodic) patterns of light for illumination of the sample plane and relies on the generation of interference patterns known as Moiré fringes. Several images are acquired under slightly different illumination conditions, e.g., by shifting and/or rotating the pattern of the structured illumination, to create the Moiré fringes. Mathematical deconvolution of the resulting interference signal allows reconstruction of a super-resolution image having up to about a two-fold improvement in spatial resolution over that achieved using diffraction-limited imaging optics [Lutz (2011), "Biological Imaging by Superresolution Light Microscopy", *Comprehensive Biotechnology (Second Ed.)*, vol. 1, pages 579-589, Elsevier; Feiner-Gracia, et al. (2018), "15—Advanced Optical Microscopy Techniques for the Investigation of Cell-Nanoparticle Interactions", *Smart Nanoparticles for Biomedicine: Micro and Nano Technologies*, pages 219-236, Elsevier; Nylk, et al. (2019), "Light-Sheet Fluorescence Microscopy With Structured Light", *Neurophotonics and Biomedical Spectroscopy*, pages 477-501, Elsevier]. An example of structured illumination microscopy imaging systems has recently been described in Hong, U.S. Patent Application Publication No. 2020/0218052.

FIG. 41 provides a non-limiting schematic illustration of an imaging system 4100 comprising a branched structured illumination optical design as disclosed herein. The first branch (or arm) of the illumination optical path of system 4100 comprises, e.g., a light source (light emitter) 4110A, an optical collimator 4120A to collimate light emitted by light source 4110A, a diffraction grating 4130A in a first orientation with respect to the optical axis, a rotating window 4140A, and a lens 4150A. The second branch of the illumination optical path of system 4100 comprises, e.g., a light source 4110B, an optical collimator 4120B to collimate light emitted by light source 4110B, a diffraction grating 4130B in a second orientation with respect to the optical axis, a rotating window 4140B, and a lens 4150B. The diffraction gratings 4130A and 4130B enable projection of patterns of light fringes on the sample plane.

In some instances, the light sources 4110A and 4110B may be incoherent light sources (e.g., comprising one or more light emitting diodes (LEDs)) or coherent light sources (e.g., comprising one or more lasers or laser diodes). In some instances, the light sources 4110A and 4110B may comprise an optical fiber coupled to, e.g., an LED, laser, or laser diode that outputs a light beam that is then collimated by the respective collimator lenses 4120A and 4120B. In some instances, light sources 4110A and 4110B may output light

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of the same wavelength. In some instances, light sources **4110A** and **4110B** may output light of different wavelengths. Either of light sources **4110A** and **4110B** may be configured to output light of any wavelength and/or wavelength range described elsewhere herein. During imaging, light sources **4110A** and **4110B** may be switched on or off using, for example, a high-speed shutter (not shown) positioned in the optical path or by pulsing the light sources at a predetermined frequency.

In the example shown in FIG. **41**, the first illumination arm of system **4100** includes a fixed vertical grating **4130A** used to project a grating pattern (e.g., a vertical light fringe pattern) in a first orientation onto the sample plane, e.g., a first interior surface **4188** of a flow cell **4187**, and the second illumination arm includes a fixed horizontal grating **4130B** to project a grating pattern (e.g., a horizontal light fringe pattern) in a second orientation onto the sample plane **4188**. Advantageously, the diffraction gratings of imaging system **4100** do not need to be mechanically rotated or translated during imaging in this non-limiting example, which may provide improved imaging speed, system reliability, and system repeatability. In some instances, diffraction gratings **4130A** and/or **4130B** may be rotatable about their respective optical axes such that the angle between the light fringe patterns projected on the sample plane is adjustable.

As illustrated in FIG. **41**, in some instances, diffraction gratings **4130A** and **4130B** may be transmissive diffraction gratings that comprise a plurality of diffracting elements (e.g., parallel slits or grooves) formed in a glass substrate or other suitable surface. In some instances, the gratings may be implemented as phase gratings that provide a periodic variation of the refractive index of the grating material. In some instances, the groove or feature spacing may be chosen to diffract light at suitable angles and/or be tuned to the minimum resolvable feature size of the imaged samples for operation of imaging system **4100**. In other instances, the diffraction gratings may be reflective diffraction gratings.

In the example illustrated in FIG. **41**, the orientations of the vertical and horizontal light fringe patterns are offset by about 90 degrees. In other instances, other orientations of the diffraction gratings may be used to create an offset of about 90 degrees. For example, the diffraction gratings may be oriented such that they project light fringe patterns that are offset ± 45 degrees from the x or y axes of sample plane (e.g., first interior flow cell surface) **4188**. The configuration of imaging system **4100** illustrated in FIG. **41** may be particularly advantageous in the case of a sample support surface (e.g., an interior surface **4188** of a flow cell **4187**) comprising regularly patterned features laid out on a rectangular grid, as enhancement of image resolution using the structured illumination approach can be achieved using only two perpendicular grating orientations (e.g., the vertical grating orientation and horizontal grating orientation).

Diffraction gratings **4130A** and **4130B**, in the example of system **4100**, may be configured to diffract the input illumination light beams into a series of intensity maxima due to constructive interference according to the relationship:

$$m = \text{order number} = d \sin(\theta) / \lambda.$$

where d = the distance between slits or grooves in the diffraction grating, θ = the angle of incidence of the illumination light relative to a normal to the surface of the diffraction grating, λ = the wavelength of the illumination light, and m = an integer value corresponding to an intensity maxima of the diffracted light, e.g., $m=0, \pm 1, \pm 2$, etc. In some instances, a specific order of the diffracted illumination light, e.g., the first order ($m=\pm 1$) light may be projected on

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the sample plane, e.g., interior flow cell surface **4188**. In some instances, for example, vertical grating **4130A** may diffract a collimated light beam into first order diffracted beams (± 1 orders) which are focused onto the sample plane in a first orientation, and horizontal grating **4130B** may diffract a collimated light beam into first order diffracted beams which are focused onto the sample plane in a second orientation. In some instances, the zeroth order beam and/or all other higher order beams (e.g., $m=\pm 2$ or higher) may be blocked, i.e., filtered out of the illumination pattern projected onto the sample plane **4188**, using, for example, a beam blocking element (not shown) such as an order filter that may be inserted into the optical paths following the diffraction gratings.

Each branch of the structured illumination system in the example of **4100** includes an optical phase modulator or phase shifter **4140A** and **4140B** to phase shift the diffracted light transmitted or reflected by each of the diffraction gratings **4130A** and **4130B**. During structured imaging, the optical phase of each diffracted beam may be shifted by some fraction (e.g., $1/2, 1/3, 1/4$, etc.) of the pitch (X) of each fringe of the structured pattern. In the example of FIG. **41**, phase modulators **4140A** and **4140B** may be implemented, e.g., as rotating optical phase plates actuated by rotatory actuators or other actuator mechanisms to rotate and modulate the optical path-length of each diffracted beam. For example, optical phase plate **4140A** may be rotated about the vertical axis to shift the image projected by vertical grating **4130A** on sample plane **4188** left or right, and optical phase plate **4140B** may rotate about the horizontal axis to shift the image projected by horizontal grating **4130B** on sample plane **4188** in the perpendicular direction.

In other implementations, other types of phase modulators that change the optical path length of the diffracted light (e.g., optical wedges mounted on linear translation stages, etc.) may be used. Additionally, although optical phase modulators **4140A** and **4140B** are illustrated as being placed after diffraction gratings **4130A** and **4130B**, in other implementations they may be placed at other positions in the illumination optical path. In some instances, a single optical phase modulator may be operated in two different directions to produce different light fringe patterns, or the position of a single optical phase modulator may be adjusted using a single motion to simultaneously adjust the path lengths of both arms of the illumination optical path.

In the example illustrated in FIG. **41**, optical component **4160** may be used to combine light from the two illumination optical paths. Optical component **4160** may comprise, for example, a partially-silvered mirror, a dichroic mirror (depending on the wavelengths of light output by light sources **4110A** and **4110B**), a mirror comprising a pattern of holes or a patterned reflective coating such that light from the two arms of the illumination system are combined in a lossless or nearly lossless manner (e.g., without significant loss of optical power other than a small amount of absorption by the reflective coating), a polarizing beam splitter (in the case that light sources **4110A** and **4110B** are configured to produce polarized light), and the like. Optical component **4160** may be located such that the desired diffracted orders of light reflected or transmitted by each of the diffraction gratings are spatially resolved, and the unwanted orders of light are blocked. In some instances, optical component **4160** may pass the first order light output by the first illumination light path and reflect the first order light output by the second illumination light path. In some instances, the structured illumination pattern on the sample surface **4188** may be switched from a vertical orientation (e.g., using

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diffraction grating **4130A**) to a horizontal orientation (e.g., using diffraction grating **4130B**) by turning each light source on or off, or by opening and closing an optical shutter in the optical path for the light source. In other instances, the structured illumination pattern may be switched by using an optical switch to change the illumination optical path used to illuminate the sample plane.

Referring again to FIG. **41**, a lens **4170**, a semi-reflective mirror or dichroic mirror **4180**, and an objective **4185** may be used to focus the structured illumination light onto sample surface **4188** (e.g., the first interior surface of a flow cell **4187**). Light that is emitted by, reflected by, or scattered by the sample surface **4188** is then collected by objective **4185**, transmitted through mirror **4180**, and imaged by image sensor or camera **4195**. As noted, mirror **4180** may be a dichroic mirror to reflect structured illumination light received from each branch of the illumination optical path into objective **4185** for projection onto sample plane **4188**, and to pass through light emitted by the sample plane **4188** (e.g., fluorescent light, which is emitted at different wavelengths than the excitation light) for imaging onto image sensor **4195**.

In some instances, system **4100** may optionally comprise a custom tube lens **4190** as described elsewhere herein such that the focus of the imaging system may be shifted from the first interior surface **4188** to the second interior surface **4189** of the flow cell **4187** to enable dual surface imaging with minimal adjustment. In some instances, lens **4170** may comprise a custom tube lens as described elsewhere herein such that the focus of the illumination optical path may be shifted from the first interior surface **4188** to the second interior surface **4189** of the flow cell **4187** to enable dual surface imaging with minimal adjustment. In some instances, lens **4170** may be implemented to articulate along the optical axis to adjust the focus of the structured illumination pattern on the sample plane. In some instances, system **4100** may comprise an autofocus mechanism (not shown) to adjust focus of the illumination light and/or the focus of the image at the plane of image sensor **4195**. In some instances, the system **4100** illustrated in FIG. **41** may provide a high optical efficiency due to the absence of a polarizer in the optical path. The use of unpolarized light may or may not have a significant impact on illumination pattern contrast depending on the numerical aperture of objective **4185**.

For the sake of simplicity, some optical components of imaging system **4100** may have been omitted from FIG. **41** and the foregoing discussion. Although system **4100** is illustrated in this non-limiting example as a single channel detection system, in other instances, it may be implemented as a multi-channel detection system (e.g., using two different image sensors and appropriate optics as well as light sources that emit at two different wavelengths). Furthermore, although the illumination optical path of system **4100** is illustrated in this non-limiting example as comprising two branches, in some instances it may be implemented as comprising, e.g., three branches, four branches, or more than four branches, each of which comprises a diffraction grating at a fixed or adjustable relative orientation to each other.

In some instances, alternative illumination path optical designs may be used to create structured illumination. For example, in some instances, a single large, rotating optical phase modulator may be positioned after optical component **4160** and used in place of optical phase modulators **4140A** and **4140B** to modulate the phases of both diffracted beams output by the vertical and horizontal diffraction gratings **4130A** and **4130B**. In some instances, instead of being

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parallel with respect to the optical axis of one of the diffraction gratings, the axis of rotation for the single rotating optical compensator may be offset by 45 degrees (or another angular offset) from the optical axis of each of the vertical and horizontal diffraction gratings to allow for phase shifting along both illumination directions. In some instances, the single rotating optical phase modulator may be replaced by, e.g., a wedged optical component rotating about the nominal beam axis.

In another alternative illumination optical path design, diffraction gratings **4130A** and **4130B** may be mounted on respective linear motion stages so that they may be translated to change the optical path length (and thus the phase) of light reflected or transmitted by diffraction gratings **4130A** and **4130B**. The axis of motion of the linear motion stages may be perpendicular or otherwise offset from the orientation of their respective diffraction grating to provide translation of the diffraction grating's fringe pattern along sample plane **4188**. Suitable translation stages may comprise, e.g., crossed roller bearing stages, a linear motor, a high-accuracy linear encoder, and/or other linear actuator technologies to provide precise linear translation of the diffraction gratings.

FIG. **42** provides a non-limiting example of a workflow for acquiring and processing imaged using structured illumination to enhance the spatial resolution of the imaging system. In some instances, the workflow illustrated in FIG. **42** may be performed to image an entire sample plane (e.g., an interior surface of a flow cell by means of image tiling) or to image a single area of a larger sample plane. The vertical **4130A** and horizontal **4130B** diffraction gratings of the system **4100** illustrated in FIG. **41** may be used to project illumination light fringe patterns onto the sample plane that have different known orientations and/or different known phase shifts. For example, the imaging system **4100** may use vertical grating **4130A** and horizontal grating **4130B** to generate the horizontal and vertical illumination patterns respectively, while optical phase modulators **4140A** and **4140B** may be set to three different positions to produce the three phase shifts shown for each orientation.

During operation, a first illumination condition (e.g., a specific orientation of the diffraction grating and phase shift setting) may be used to project a grating light fringe pattern on the sample plane, e.g., flow cell surface. Following capture of an image using the first illumination condition, one or more additional images acquired using one or more phase shifted illumination patterns (e.g., 1, 2, 3, 4, 5, 6, or more than 6 additional images acquired using 1, 2, 3, 4, 5, 6, or more than 6 phase shifted illumination patterns) may be acquired. If the imaging system comprises a second branch of the illumination optical path, the image acquisition process may be repeated using a second illumination condition as a starting point (e.g., a second specific orientation of the diffraction grating and phase shift setting), and the image acquisition process may be repeated. In some instances, images may be acquired for at least three different orientations of the diffraction grating (e.g., spaced apart by 60 degrees relative to each other) using at least 5 different phase shifted light fringe patterns. If no more images are to be acquired using different orientations of the diffraction grating or phase shifted illumination light fringe patterns, an image reconstruction algorithm may be used to process the acquired images and produce a reconstructed super-resolution image. In some instances, images may be acquired for at least 1, 2, 3, 4, 5, 6, or more than 6 different orientations

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of the diffraction grating using at least 1, 2, 3, 4, 5, 6, or more than 6 different phase-shifted light fringe patterns at each orientation.

A potential disadvantage of acquiring multiple images for use in reconstructing single, super-resolution images is the time required to adjust the orientation and/or relative phase shift of the projected light fringe patterns and the exposure time required for acquiring each image, as well as the downstream image processing. Therefore, optical designs that minimize the time required to change diffraction grating orientation and relative phase, along with highly efficient image reconstruction algorithms, are to be preferred. In some instances, fewer images may be required to reconstruct super-resolution images of, e.g., flow cell surfaces comprising discrete, fluorescently labeled clusters of amplified target nucleic acid sequences tethered to the low-nonspecific binding surfaces described elsewhere herein than would ordinarily be required for reconstructing higher resolution images of conventional samples, e.g., stained tissue samples.

Referring again to FIG. 42, the afore-mentioned cycle may be repeated for different areas of a given flow cell surface, e.g., in the case that the images will be tiled to create a higher resolution image of the entire flow cell surface. In some instances, the afore-mentioned cycle may be repeated after adjusting the focus of the imaging system if, e.g., a second flow cell surface is to be imaged.

Other Super-Resolution Imaging Techniques:

In some instances, the disclosed imaging systems may comprise the use of an alternative super-resolution imaging technique, e.g., photoactivation localization microscopy (PALM), fluorescence photoactivation localization microscopy (FPALM), and/or stochastic optical reconstruction microscopy (STORM) [see, for example, Lutz, et al. (2011), "Biological Imaging by Superresolution Light Microscopy", *Comprehensive Biotechnology (Second Ed.)*, vol. 1, pages 579-589, Elsevier), which are based on statistical curve fitting of the intensity distribution observed in images of a single molecule's point spread function (PSF) to a Gaussian distribution function. The Gaussian distribution function is then used to define location of the molecule in the sample plane with much higher precision than allowed by the classical resolution limit. The same approach may be used to image, e.g., small dispersed subsets of fluorescently labeled molecules such as clonally amplified clusters of target nucleic acid sequences tethered to a low non-specific binding surface on a sample support or the interior surface of a flow cell.

The spatial accuracy or resolution achieved using these methods depends upon the number of photons collected from the molecule before it is photobleached and upon the background noise level [Lutz, et al. (2011), *ibid*]. In the case that background noise is negligible and collection of at least 10,000 photons per molecule is possible, position accuracies of 1-2 nm have been demonstrated. In some instances, e.g., using the sequencing-by-avidity approach described elsewhere herein, polymer-nucleotide conjugates comprising a plurality of fluorescent labels (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 labels per conjugate) to ensure a high photon count, optionally used in combination with the low non-specific binding surfaces disclosed elsewhere herein to ensure very low background signals, may facilitate the use of these super-resolution imaging techniques for genetic testing and sequencing applications. Spatial accuracy or resolution decreases with decreasing numbers of photons collected, however, even in the case that only moderate numbers of photons are collected, position location accuracy or resolution of 20 nm is possible. In some cases, an

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improvement of 10-fold or better in lateral spatial resolution may be achieved. In some cases, an image resolution of better than 500 nm, 400 nm, 300 nm, 200 nm, 175 nm, 150 nm, 125 nm, 100 nm, 75 nm, 50 nm, 25 nm, or 10 nm may be achieved.

The second principle fundamental to this class of imaging is that small numbers of spatially separated fluorescent molecules within the sample are imaged at any given time.

In some instances, the ability to control fluorescence emission of small, dispersed subsets of fluorescent molecules in the sample plane is key to facilitating super-resolution imaging. In the case of fluorescence photoactivation localization microscopy (FPALM) and photoactivation localization microscopy (PALM), for example, the use of photoactivatable green fluorescent proteins (PA-GFP) as a label has allowed for controlled induction of fluorescent subsets in a sample using short pulses of 405 nm light to photoconvert the PA-GFP from a dark, nonfluorescent state to a 488 nm excitable fluorescent state, thereby resulting in spatially separated subsets of fluorescent molecules that can be imaged [Lutz, et al. (2011), *ibid*]. In the case of stochastic optical reconstruction microscopy (STORM), the photo-switching properties of, for example, the cyanine dye pairs Cy5-Cy3 may be used in a similar fashion to enable the stochastic induction of Cy5 fluorescence from a small subset of the molecules in the sample at any given time, e.g., small subsets of molecules that are spatially separated by at least several resolution units. In some instances, e.g., when combined with the sequencing-by-avidity approach described elsewhere herein, polymer-nucleotide conjugates may comprise a photoactivatable green fluorescent protein (PA-GFP) or a subdomain or portion thereof. In some instances, the polymer-nucleotide conjugates may comprise a mixture of conjugates in which a first portion is labeled with, e.g., Cy3 labels, and a second portion is labeled with, e.g., Cy5 labels. In some instances, the polymer nucleotide conjugates may comprise a mixture of, e.g., Cy3 and Cy5 labels within the same conjugate.

The super-resolved image is reconstructed from the sum of the Gaussian fits from all molecules or features (e.g., labeled nucleic acid clusters) imaged in a time stack of acquired images [Lutz, et al. (2011), *ibid*], where the intensity corresponds to the positional uncertainty of the location of each molecule or subset of molecules. Unique to this kind of data set is the ability to render the image with different localization precisions or resolutions. In some instances, an imaging module comprising a total internal reflectance fluorescence (TIRF) optical imaging design may be advantageous in implementing the use of these super-resolution imaging techniques as the evanescent wave used for excitation of fluorescence is restricted in the axial dimension to less than 200 nm from the sample support or flow cell surface and thus suppresses background fluorescence signal. In some instances, the imaging system may comprise a higher numerical aperture objective than utilized in other imaging module designs disclosed herein. The use of higher numerical aperture objectives may facilitate implementation of evanescent wave excitation and highly efficient capture of photons from the fluorescent probes. In some instances, wide-field imaging using single-photon-sensitive EM-CCD cameras or other types of image sensors may enable simultaneous imaging of many molecules or subsets of molecules (e.g., nucleic acid sequence clusters) per frame, thereby improving the throughput of image acquisition.

In some instances, the data acquisition time required to acquire enough images for adequate feature definition and resolution may be shortened by improvements in the sensi-

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tivity and speed of the imaging system, through the use of the sequencing-by-avidity reagents and low non-specific binding surfaced disclosed herein to increase signal while reducing or eliminating background, and the use of improved image reconstruction algorithms.

Assessing Image Quality:

For any of the embodiments of the optical imaging designs disclosed herein, imaging performance or imaging quality may be assessed using any of a variety of performance metrics known to those of skill in the art. Examples include, but are not limited to, measurements of modulation transfer function (MTF) at one or more specified spatial frequencies, defocus, spherical aberration, chromatic aberration, coma, astigmatism, field curvature, image distortion, contrast-to-noise ratio (CNR), or any combination thereof.

In some instances, the disclosed optical designs for dual-side imaging (e.g., the disclosed objective lens designs, tube lens designs, the use of an electro-optical phase plate in combination with an objective, etc., alone or in combination) may yield significant improvements for image quality for both the upper (near) and lower (far) interior surfaces of a flow cell, such that the difference in an imaging performance metric for imaging the upper interior surface and the lower interior surface of the flow cell is less than 20%, less than 15%, less than 10%, less than 5%, less than 4%, less than 3%, less than 2%, or less than 1% for any of the imaging performance metrics listed above, either individually or in combination.

In some instances, the disclosed optical designs for dual-side imaging (e.g., comprising the disclosed tube lens designs, the use of an electro-optical phase plate in combination with an objective, etc.) may yield significant improvements for image quality such that an image quality performance metric for dual-side imaging provides for an at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, or at least 30% improvement in the imaging performance metric for dual-side imaging compared to that for a conventional system comprising, e.g., an objective lens, a motion-actuated compensator (that is moved out of or into the optical path when imaging the near or far interior surfaces of a flow cell), and an image sensor for any of the imaging performance metrics listed above, either individually or in combination. In some instances, fluorescence imaging systems comprising one or more of the disclosed tube lens designs provides for an at least equivalent or better improvement in an imaging performance metric for dual-side imaging compared to that for a conventional system comprising an objective lens, a motion-actuated compensator, and an image sensor. In some instances, fluorescence imaging systems comprising one or more of the disclosed tube lens designs provides for an at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% improvement in an imaging performance metric for dual-side imaging compared to that for a conventional system comprising an objective lens, a motion-actuated compensator, and an image sensor.

Imaging Module Specifications:

Excitation Light Wavelength(s):

In any of the disclosed optical imaging module designs, the light source(s) of the disclosed imaging modules may produce visible light, such as green light and/or red light. In some instances, the light source(s), alone or in combination with one or more optical components, e.g., excitation optical filters and/or dichroic beam splitters, may produce excitation light at about 350 nm, 375 nm, 400 nm, 425 nm, 450 nm, 475 nm, 500 nm, 525 nm, 550 nm, 575 nm, 600 nm, 625 nm, 650 nm, 675 nm, 700 nm, 725 nm, 750 nm, 775 nm, 800 nm,

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825 nm, 850 nm, 875 nm, or 900 nm. Those of skill in the art will recognize that the excitation wavelength may have any value within this range, e.g., about 620 nm.

Excitation Light Bandwidths:

In any of the disclosed optical imaging module designs, the light source(s), alone or in combination with one or more optical components, e.g., excitation optical filters and/or dichroic beam splitters, may produce light at the specified excitation wavelength within a bandwidth of ± 2 nm, ± 5 nm, ± 10 nm, ± 20 nm, ± 40 nm, ± 80 nm, or greater. Those of skill in the art will recognize that the excitation bandwidths may have any value within this range, e.g., about ± 18 nm.

Light Source Power Output:

In any of the disclosed optical imaging module designs, the output of the light source(s) and/or an excitation light beam derived therefrom (including a composite excitation light beam) may range in power from about 0.5 W to about 5.0 W, or more (as will be discussed in more detail below). In some instances, the output of the light source and/or the power of an excitation light beam derived therefrom may be at least 0.5 W, at least 0.6 W, at least 0.7 W, at least 0.8 W, at least 1 W, at least 1.1 W, at least 1.2 W, at least 1.3 W, at least 1.4 W, at least 1.5 W, at least 1.6 W, at least 1.8 W, at least 2.0 W, at least 2.2 W, at least 2.4 W, at least 2.6 W, at least 2.8 W, at least 3.0 W, at least 3.5 W, at least 4.0 W, at least 4.5 W, or at least 5.0 W. In some implementations, the output of the light source and/or the power of an excitation light beam derived therefrom (including a composite excitation light beam) may be at most 5.0 W, at most 4.5 W, at most 4.0 W, at most 3.5 W, at most 3.0 W, at most 2.8 W, at most 2.6 W, at most 2.4 W, at most 2.2 W, at most 2.0 W, at most 1.8 W, at most 1.6 W, at most 1.5 W, at most 1.4 W, at most 1.3 W, at most 1.2 W, at most 1.1 W, at most 1 W, at most 0.8 W, at most 0.7 W, at most 0.6 W, or at most 0.5 W. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the output of the light source and/or the power of an excitation light beam derived therefrom (including a composite excitation light beam) may range from about 0.8 W to about 2.4 W. Those of skill in the art will recognize that the output of the light source and/or the power of an excitation light beam derived therefrom (including a composite excitation light beam) may have any value within this range, e.g., about 1.28 W.

Light Source Output Power and CNR:

In some implementations of the disclosed optical imaging module designs, the output power of the light source(s) and/or the power of excitation light beam(s) derived therefrom (including a composite excitation light beam) is sufficient, in combination with an appropriate sample, to provide for a contrast-to-noise ratio (CNR) in images acquired by the illumination and imaging module of at least 5, at least 10, at least 15, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 30, at least 35, at least 40, or at least 50 or more, or any CNR within any range formed by any of these values.

Fluorescence Emission Bands:

In some instances, the disclosed fluorescence optical imaging modules may be configured to detect fluorescence emission produced by any of a variety of fluorophores known to those of skill in the art. Examples of suitable fluorescence dyes for use in, e.g., genotyping and nucleic acid sequencing applications (e.g., by conjugation to nucleotides, oligonucleotides, or proteins) include, but are not limited to, fluorescein, rhodamine, coumarin, cyanine, and

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derivatives thereof, including the cyanine derivatives cyanine dye-3 (Cy3), cyanine dye-5 (Cy5), cyanine dye-7 (Cy7), etc.

Fluorescence Emission Wavelengths:

In any of the disclosed optical imaging module designs, the detection channel or imaging channel of the disclosed optical systems may include one or more optical components, e.g., emission optical filters and/or dichroic beam splitters, configured to collect emission light at about 350 nm, 375 nm, 400 nm, 425 nm, 450 nm, 475 nm, 500 nm, 525 nm, 550 nm, 575 nm, 600 nm, 625 nm, 650 nm, 675 nm, 700 nm, 725 nm, 750 nm, 775 nm, 800 nm, 825 nm, 850 nm, 875 nm, or 900 nm. Those of skill in the art will recognize that the emission wavelength may have any value within this range, e.g., about 825 nm.

Fluorescence Emission Light Bandwidths:

In any of the disclosed optical imaging module designs, the detection channel or imaging channel may comprise one or more optical components, e.g., emission optical filters and/or dichroic beam splitters, configured to collect light at the specified emission wavelength within a bandwidth of ± 2 nm, ± 5 nm, ± 10 nm, ± 20 nm, ± 40 nm, ± 80 nm, or greater. Those of skill in the art will recognize that the excitation bandwidths may have any value within this range, e.g., about ± 18 nm.

Numerical Aperture:

In some instances, the numerical aperture of the objective lens and/or optical imaging module (e.g., comprising an objective lens and/or tube lens) in any of the disclosed optical system designs may range from about 0.1 to about 1.4. In some instances, the numerical aperture may be at least 0.1, at least 0.2, at least 0.3, at least 0.4, at least 0.5, at least 0.6, at least 0.7, at least 0.8, at least 0.9, at least 1.0, at least 1.1, at least 1.2, at least 1.3, or at least 1.4. In some instances, the numerical aperture may be at most 1.4, at most 1.3, at most 1.2, at most 1.1, at most 1.0, at most 0.9, at most 0.8, at most 0.7, at most 0.6, at most 0.5, at most 0.4, at most 0.3, at most 0.2, or at most 0.1. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the numerical aperture may range from about 0.1 to about 0.6. Those of skill in the art will recognize that the numerical aperture may have any value within this range, e.g., about 0.55.

Optical Resolution:

In some instances, depending on the numerical aperture of the objective lens and/or optical system (e.g., comprising an objective lens and/or tube lens), the minimum resolvable spot (or feature) separation distance at the sample plane achieved by any of the disclosed optical system designs may range from about 0.5 μm to about 2 μm . In some instances, the minimum resolvable spot separation distance at the sample plane may be at least 0.5 μm , at least 0.6 μm , at least 0.7 μm , at least 0.8 μm , at least 0.9 μm , at least 1.0 μm , at least 1.2 μm , at least 1.4 μm , at least 1.6 μm , at least 1.8 μm , or at least 1.0 μm . In some instances, the minimum resolvable spot separation distance may be at most 2.0 μm , at most 1.8 μm , at most 1.6 μm , at most 1.4 μm , at most 1.2 μm , at most 1.0 μm , at most 0.9 μm , at most 0.8 μm , at most 0.7 μm , at most 0.6 μm , or at most 0.5 μm . Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the minimum resolvable spot separation distance may range from about 0.8 μm to about 1.6 μm . Those of skill in the art will recognize that the minimum resolvable spot separation distance may have any value within this range, e.g., about 0.95 μm .

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Optical Resolution of First and Second Surfaces at Different Depths:

In some instances, the use of the novel objective lens and/or tube lens designs disclosed herein, in any of the optical modules or systems disclosed herein, may confer comparable optical resolution for first and second surfaces (e.g. the upper and lower interior surfaces of a flow cell) with or without the need to refocus between acquiring the images of the first and second surfaces. In some instances, the optical resolution of the images thus obtained of the first and second surfaces may be with 20%, 18%, 16%, 14%, 12%, 10%, 8%, 6%, 4%, 2%, or 1% of each other, or within any value within this range.

Magnification:

In some instances, the magnification of the objective lens and/or tube lens, and/or optical system (e.g., comprising an objective lens and/or tube lens) in any of the disclosed optical configurations may range from about 2 \times to about 20 \times . In some instances, the optical system magnification may be at least 2 \times , at least 3 \times , at least 4 \times , at least 5 \times , at least 6 \times , at least 7 \times , at least 8 \times , at least 9 \times , at least 10 \times , at least 15 \times , or at least 20 \times . In some instances, the optical system magnification may be at most 20 \times , at most 15 \times , at most 10 \times , at most 9 \times , at most 8 \times , at most 7 \times , at most 6 \times , at most 5 \times , at most 4 \times , at most 3 \times , or at most 2 \times . Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the optical system magnification may range from about 3 \times to about 10 \times . Those of skill in the art will recognize that the optical system magnification may have any value within this range, e.g., about 7.5 \times .

Objective Lens Focal Length:

In some implementations of the disclosed optical designs, the focal length of the objective lens may range between 20 mm and 40 mm. In some instances, the focal length of the objective lens may be at least 20 mm, at least 25 mm, at least 30 mm, at least 35 mm, or at least 40 mm. In some instances, the focal length of the objective lens may be at most 40 mm, at most 35 mm, at most 30 mm, at most 25 mm, or at most 20 mm. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the focal length of the objective lens may range from 25 mm to 35 mm. Those of skill in the art will recognize that the focal length of the objective lens may have any value within the range of values specified above, e.g., about 37 mm.

Objective Lens Working Distance:

In some implementations of the disclosed optical designs, the working distance of the objective lens may range between about 100 μm and 30 mm. In some instances, the working distance may be at least 100 μm , at least 200 μm , at least 300 μm , at least 400 μm , at least 500 μm , at least 600 μm , at least 700 μm , at least 800 μm , at least 900 μm , at least 1 mm, at least 2 mm, at least 4 mm, at least 6 mm, at least 8 mm, at least 10 mm, at least 15 mm, at least 20 mm, at least 25 mm, or at least 30 mm. In some instances, the working distance may be at most 30 mm, at most 25 mm, at most 20 mm, at most 15 mm, at most 10 mm, at most 8 mm, at most 6 mm, at most 4 mm, at most 2 mm, at most 1 mm, at most 900 μm , at most 800 μm , at most 700 μm , at most 600 μm , at most 500 μm , at most 400 μm , at most 300 μm , at most 200 μm , at most 100 μm . Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the working distance of the objective lens may range from 500 μm to 2 mm. Those of skill in the art

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will recognize that the working distance of the objective lens may have any value within the range of values specified above, e.g., about 1.25 mm.

Objectives Optimized for Imaging Through Thick Coverslips:

In some instances of the disclosed optical designs, the design of the objective lens may be improved or optimized for a different coverslip of flow cell thickness. For example, in some instances the objective lens may be designed for optimal optical performance for a coverslip that is from about 200 μm to about 1,000 μm thick. In some instances, the objective lens may be designed for optimal performance with a coverslip that is at least 200 μm , at least 300 μm , at least 400 μm , at least 500 μm , at least 600 μm , at least 700 μm , at least 800 μm , at least 900 μm , or at least 1,000 μm thick. In some instances, the objective lens may be designed for optimal performance with a coverslip that is at most 1,000 μm , at most 900 μm , at most 800 μm , at most 700 μm , at most 600 μm , at most 500 μm , at most 400 μm , at most 300 μm , or at most 200 μm thick. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the objective lens may be designed for optimal optical performance for a coverslip that may range from about 300 μm to about 900 μm . Those of skill in the art will recognize that the objective lens may be designed for optimal optical performance for a coverslip that may have any value within this range, e.g., about 725 μm .

Depth of Field and Depth of Focus:

In some instances, the depth of field and/or depth of focus for any of the disclosed imaging module (e.g., comprising an objective lens and/or tube lens) designs may range from about 10 μm to about 800 μm , or more. In some instances, the depth of field and/or depth of focus may be at least 10 μm , at least 20 μm , at least 30 μm , at least 40 μm , at least 50 μm , at least 75 μm , at least 100 μm , at least 125 μm , at least 150 μm , at least 175 μm , at least 200 μm , at least 250 μm , at least 300 μm , at least 300 μm , at least 400 μm , at least 500 μm , at least 600 μm , at least 700 μm , or at least 800 μm , or more. In some instances, the depth of field and/or depth of focus be at most 800 μm , at most 700 μm , at most 600 μm , at most 500 μm , at most 400 μm , at most 300 μm , at most 250 μm , at most 200 μm , at most 175 μm , at most 150 μm , at most 125 μm , at most 100 μm , at most 75 μm , at most 50 μm , at most 40 μm , at most 30 μm , at most 20 μm , at most 10 μm , or less. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the depth of field and/or depth of focus may range from about 100 μm to about 175 μm . Those of skill in the art will recognize that the depth of field and/or depth of focus may have any value within the range of values specified above, e.g., about 132 μm .

Field of View (FOV):

In some implementations, the FOV of any of the disclosed imaging module designs (e.g., that provided by a combination of objective lens and detection channel optics (such as a tube lens)) may range, for example, between about 1 mm and 5 mm (e.g., in diameter, width, length, or longest dimension). In some instances, the FOV may be at least 1.0 mm, at least 1.5 mm, at least 2.0 mm, at least 2.5 mm, at least 3.0 mm, at least 3.5 mm, at least 4.0 mm, at least 4.5 mm, or at least 5.0 mm (e.g., in diameter, width, length, or longest dimension). In some instances, the FOV may be at most 5.0 mm, at most 4.5 mm, at most 4.0 mm, at most 3.5 mm, at most 3.0 mm, at most 2.5 mm, at most 2.0 mm, at most 1.5 mm, or at most 1.0 mm (e.g., in diameter, width, length, or

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longest dimension). Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the FOV may range from about 1.5 mm to about 3.5 mm (e.g., in diameter, width, length, or longest dimension). Those of skill in the art will recognize that the FOV may have any value within the range of values specified above, e.g., about 3.2 mm (e.g., in diameter, width, length, or longest dimension).

Field-of-View (FOV) Area:

In some instances of the disclosed optical system designs, the area of the field-of-view may range from about 2 mm^2 to about 5 mm^2 . In some instances, the field-of-view may be at least 2 mm^2 , at least 3 mm^2 , at least 4 mm^2 , or at least 5 mm^2 in area. In some instances, the field-of-view may be at most 5 mm^2 , at most 4 mm^2 , at most 3 mm^2 , or at most 2 mm^2 in area. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the field-of-view may range from about 3 mm^2 to about 4 mm^2 in area. Those of skill in the art will recognize that the area of the field-of-view may have any value within this range, e.g., 2.75 mm^2 .

Optimization of Objective Lens and/or Tube Lens MTF:

In some instances, the design of the objective lens and/or at least one tube lens in the disclosed imaging modules and systems is configured to optimize the modulation transfer function in the mid to high spatial frequency range. For example, in some instances, the design of the objective lens and/or at least one tube lens in the disclosed imaging modules and systems is configured to optimize the modulation transfer function in the spatial frequency range from 500 cycles per mm to 900 cycles per mm, from 700 cycles per mm to 1100 cycles per mm, from 800 cycles per mm to 1200 cycles per mm, or from 600 cycles per mm to 1000 cycles per mm in the sample plane.

Optical Aberration and Diffraction-Limited Imaging Performance:

In some implementations of any of the optical imaging module designs disclosed herein, the objective lens and/or tube lens may be configured to provide the imaging module with a field-of-view as indicated above such that the FOV has less than 0.15 waves of aberration over at least 60%, 70%, 80%, 90%, or 95% of the field. In some implementations, the objective lens and/or tube lens may be configured to provide the imaging module with a field-of-view as indicated above such that the FOV has less than 0.1 waves of aberration over at least 60%, 70%, 80%, 90%, or 95% of the field. In some implementations, the objective lens and/or tube lens may be configured to provide the imaging module with a field-of-view as indicated above such that the FOV is diffraction-limited over at least 60%, 70%, 80%, 90%, or 95% of the field.

Angle of Incidence of Light Beams on Dichroic Reflectors, Beam Splitter, and Beam Combiners:

In some instances of the disclosed optical designs, the angles of incidence for a light beam incident on a dichroic reflector, beam splitter, or beam combiner may range between about 20 degrees and about 45 degrees. In some instances, the angles of incidence may be at least 20 degrees, at least 25 degrees, at least 30 degrees, at least 35 degrees, at least 40 degrees, or at least 45 degrees. In some instances, the angles of incidence may be at most 45 degrees, at most

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40 degrees, at most 35 degrees, at most 30 degrees, at most 25 degrees, or at most 20 degrees. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the angles of incidence may range from about 25 degrees to about 40 degrees. Those of skill in the art will recognize that the angles of incidence may have any value within the range of values specified above, e.g., about 43 degrees.

Image Sensor (Photodetector Array) Size:

In some instances, the disclosed optical systems may comprise image sensor(s) having an active area with a diagonal ranging from about 10 mm to about 30 mm, or larger. In some instances, the image sensors may have an active area with a diagonal of at least 10 mm, at least 12 mm, at least 14 mm, at least 16 mm, at least 18 mm, at least 20 mm, at least 22 mm, at least 24 mm, at least 26 mm, at least 28 mm, or at least 30 mm. In some instances, the image sensors may have an active area with a diagonal of at most 30 mm, at most 28 mm, at most 26 mm, at most 24 mm, at most 22 mm, at most 20 mm, at most 18 mm, at most 16 mm, at most 14 mm, at most 12 mm, or at most 10 mm. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the image sensor(s) may have an active area with a diagonal ranging from about 12 mm to about 24 mm. Those of skill in the art will recognize that the image sensor(s) may have an active area with a diagonal having any value within the range of values specified above, e.g., about 28.5 mm.

Image Sensor Pixel Size and Pitch:

In some instances, the pixel size and/or pitch selected for the image sensor(s) used in the disclosed optical system designs may range in at least one dimension from about 1 μm to about 10 μm . In some instances, the pixel size and/or pitch may be at least 1 μm , at least 2 μm , at least 3 μm , at least 4 μm , at least 5 μm , at least 6 μm , at least 7 μm , at least 8 μm , at least 9 μm , or at least 10 μm . In some instances, the pixel size and/or pitch may be at most 10 μm , at most 9 μm , at most 8 μm , at most 7 μm , at most 6 μm , at most 5 μm , at most 4 μm , at most 3 μm , at most 2 μm , or at most 1 μm . Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the pixel size and/or pitch may range from about 3 μm to about 9 μm . Those of skill in the art will recognize that the pixel size and/or pitch may have any value within this range, e.g., about 1.4 μm .

Oversampling:

In some instances of the disclosed optical designs, a spatial oversampling scheme is utilized wherein the spatial sampling frequency is at least 2 \times , 2.5 \times , 3 \times , 3.5 \times , 4 \times , 4.5 \times , 5 \times , 6 \times , 7 \times , 8 \times , 9 \times , or 10 \times the optical resolution X (lp/mm).

Maximum Translation Stage Velocity:

In some instances of the disclosed optical imaging modules, the maximum translation stage velocity on any one axis may range from about 1 mm/sec to about 5 mm/sec. In some instances, the maximum translation stage velocity may be at least 1 mm/sec, at least 2 mm/sec, at least 3 mm/sec, at least 4 mm/sec, or at least 5 mm/sec. In some instances, the maximum translation stage velocity may be at most 5 mm/sec, at most 4 mm/sec, at most 3 mm/sec, at most 2 mm/sec, or at most 1 mm/sec. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the maximum translation stage velocity may range from about 2 mm/sec to about 4 mm/sec. Those

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of skill in the art will recognize that the maximum translation stage velocity may have any value within this range, e.g., about 2.6 mm/sec.

Maximum Translation Stage Acceleration:

In some instances of the disclosed optical imaging modules, the maximum acceleration on any one axis of motion may range from about 2 mm/sec² to about 10 mm/sec². In some instances, the maximum acceleration may be at least 2 mm/sec², at least 3 mm/sec², at least 4 mm/sec², at least 5 mm/sec², at least 6 mm/sec², at least 7 mm/sec², at least 8 mm/sec², at least 9 mm/sec², or at least 10 mm/sec². In some instances, the maximum acceleration may be at most 10 mm/sec², at most 9 mm/sec², at most 8 mm/sec², at most 7 mm/sec², at most 6 mm/sec², at most 5 mm/sec², at most 4 mm/sec², at most 3 mm/sec², or at most 2 mm/sec². Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the maximum acceleration may range from about 2 mm/sec² to about 8 mm/sec². Those of skill in the art will recognize that the maximum acceleration may have any value within this range, e.g., about 3.7 mm/sec².

Translation Stage Positioning Repeatability:

In some instances of the disclosed optical imaging modules, the repeatability of positioning for any one axis may range from about 0.1 μm to about 2 μm . In some instances, the repeatability of positioning may be at least 0.1 μm , at least 0.2 μm , at least 0.3 μm , at least 0.4 μm , at least 0.5 μm , at least 0.6 μm , at least 0.7 μm , at least 0.8 μm , at least 0.9 μm , at least 1.0 μm , at least 1.2 μm , at least 1.4 μm , at least 1.6 μm , at least 1.8 μm , or at least 2.0 μm . In some instances, the repeatability of positioning may be at most 2.0 μm , at most 1.8 μm , at most 1.6 μm , at most 1.4 μm , at most 1.2 μm , at most 1.0 μm , at most 0.9 μm , at most 0.8 μm , at most 0.7 μm , at most 0.6 μm , at most 0.5 μm , at most 0.4 μm , at most 0.3 μm , at most 0.2 μm , or at most 0.1 μm . Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the repeatability of positioning may range from about 0.3 μm to about 1.2 μm . Those of skill in the art will recognize that the repeatability of positioning may have any value within this range, e.g., about 0.47 μm .

FOV Repositioning Time:

In some instances of the disclosed optical imaging modules, the maximum time required to reposition the sample plane (field-of-view) relative to the optics, or vice versa, may range from about 0.1 sec to about 0.5 sec. In some instances, the maximum repositioning time (i.e., the scan stage step and settle time) may be at least 0.1 sec, at least 0.2 sec, at least 0.3 sec, at least 0.4 sec, or at least 0.5 sec. In some instances, the maximum repositioning time may be at most 0.5 sec, at most 0.4 sec, at most 0.3 sec, at most 0.2 sec, or at most 0.1 sec. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the maximum repositioning time may range from about 0.2 sec to about 0.4 sec. Those of skill in the art will recognize that the maximum repositioning time may have any value within this range, e.g., about 0.45 sec.

Error Threshold for Autofocus Correction:

In some instances of the disclosed optical imaging modules, the specified error threshold for triggering an autofocus correction may range from about 50 nm to about 200 nm. In some instances, the error threshold may be at least 50 nm, at least 75 nm, at least 100 nm, at least 125 nm, at least 150 nm, at least 175 nm, or at least 200 nm. In some instances, the

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error threshold may be at most 200 nm, at most 175 nm, at most 150 nm, at most 125 nm, at most 100 nm, at most 75 nm, or at most 50 nm. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the error threshold may range from about 75 nm to about 150 nm. Those of skill in the art will recognize that the error threshold may have any value within this range, e.g., about 105 nm.

Image Acquisition Time:

In some instances of the disclosed optical imaging modules, the image acquisition time may range from about 0.001 sec to about 1 sec. In some instances, the image acquisition time may be at least 0.001 sec, at least 0.01 sec, at least 0.1 sec, or at least 1 sec. In some instances, the image acquisition time may be at most 1 sec, at most 0.1 sec, at most 0.01 sec, or at most 0.001 sec. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the image acquisition time may range from about 0.01 sec to about 0.1 sec. Those of skill in the art will recognize that the image acquisition time may have any value within this range, e.g., about 0.250 seconds.

Imaging Time Per FOV:

In some instances, the imaging times may range from about 0.5 seconds to about 3 seconds per field-of-view. In some instances, the imaging time may be at least 0.5 seconds, at least 1 second, at least 1.5 seconds, at least 2 seconds, at least 2.5 seconds, or at least 3 seconds per FOV. In some instances, the imaging time may be at most 3 seconds, at most 2.5 seconds, at most 2 seconds, at most 1.5 seconds, at most 1 second, or at most 0.5 seconds per FOV. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the imaging time may range from about 1 second to about 2.5 seconds. Those of skill in the art will recognize that the imaging time may have any value within this range, e.g., about 1.85 seconds.

Flatness of Field:

In some instances, images across 80%, 90%, 95%, 98%, 99%, or 100% percent of the field-of-view are acquired within ± 200 nm, ± 175 nm, ± 150 nm, ± 125 nm, ± 100 nm, ± 75 nm, or ± 50 nm relative to the best focal plane for each fluorescence (or other imaging mode) detection channel.

Systems and System Components for Genomics and Other Applications:

As noted above, in some implementations, the disclosed optical imaging modules may function as modules, components, sub-assemblies, or sub-systems of larger systems configured for performing, e.g., genomics applications (e.g., genetic testing and/or nucleic acid sequencing applications) or other chemical analysis, biochemical analysis, nucleic acid analysis, cell analysis or tissue analysis applications. FIG. 39 provides a non-limiting example of a block diagram for, e.g., a sequencing system as disclosed herein. In addition to one, two, three, four, or more than four imaging modules as disclosed herein (each of which may comprise one or more illumination optical paths and/or one or more detection optical paths (e.g., one or more detection channels configured for imaging fluorescence emission within a specified wavelength range onto an image sensor)), such systems may comprise one or more X-Y translation stages, one or more X-Y-Z translation stages, flow cells or cartridges, fluidics systems and fluid flow control modules, reagent cartridges, temperature control modules, fluid dispensing robotics, cartridge- and/or microplate-handling (pick-and-place) robot-

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ics, light-tight housings and/or environmental control chambers, one or more processors or computers, data storage modules, data communication modules (e.g., Bluetooth, WiFi, intranet, or internet communication hardware and associated software), display modules, one or more local and/or cloud-based software packages (e.g., instrument/system control software packages, image processing software packages, data analysis software packages), etc., or any combination thereof.

Translation Stages:

In some implementations of the imaging and analysis systems (e.g., nucleic acid sequencing systems) disclosed herein, the system may comprise one or more (e.g., one, two, three, four, or more than four) high precision X-Y (or in some cases, X-Y-Z) translation stage(s) for re-positioning one or more sample support structure(s) (e.g., flow cell(s)) in relation to the one or more imaging modules, for example, in order to tile one or more images, each corresponding to a field-of-view of the imaging module, to reconstruct composite image(s) of an entire flow cell surface. In some implementations of the imaging systems and genomics analysis systems (e.g., nucleic acid sequencing systems) disclosed herein, the system may comprise one or more (e.g., one, two, three, four, or more than four) high precision X-Y (or in some cases, X-Y-Z) translation stage(s) for re-positioning the one or more imaging modules in relation to one or more sample support structure(s) (e.g., flow cell(s)), for example, in order to tile one or more images, each corresponding to a field-of-view of the imaging module, to reconstruct composite image(s) of an entire flow cell surface.

Suitable translation stages are commercially available from a variety of vendors, for example, Parker Hannifin. Precision translation stage systems typically comprise a combination of several components including, but not limited to, linear actuators, optical encoders, servo and/or stepper motors, and motor controllers or drive units. High precision and repeatability of stage movement is required for the systems and methods disclosed herein in order to ensure accurate and reproducible positioning and imaging of, e.g., fluorescence signals when interspersing repeated steps of reagent delivery and optical detection.

Consequently, the systems disclosed herein may comprise specifying the precision with which the translation stage is configured to position a sample support structure in relation to the illumination and/or imaging optics (or vice versa). In one aspect of the present disclosure, the precision of the one or more translation stages is between about 0.1 μ m and about 10 μ m. In other aspects, the precision of the translation stage is about 10 μ m or less, about 9 μ m or less, about 8 μ m or less, about 7 μ m or less, about 6 μ m or less, about 5 μ m or less, about 4 μ m or less, about 3 μ m or less, about 2 μ m or less, about 1 μ m or less, about 0.9 μ m or less, about 0.8 μ m or less, about 0.7 μ m or less, about 0.6 μ m or less, about 0.5 μ m or less, about 0.4 μ m or less, about 0.3 μ m or less, about 0.2 μ m or less, or about 0.1 μ m or less. Those of skill in the art will appreciate that, in some instances, the positioning precision of the translation stage may fall within any range bounded by any of two of these values (e.g. from about 0.5 μ m to about 1.5 μ m). In some instances, the positioning precision of the translation stage may have any value within the range of values included in this paragraph, e.g., about 0.12 μ m.

Flow Cells, Microfluidic Devices, and Cartridges:

The flow cell devices and flow cell cartridges disclosed herein may be used as components of systems designed for a variety of chemical analysis, biochemical analysis, nucleic

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acid analysis, cell analysis, or tissue analysis application. In general, such systems may comprise one or more one or more of the disclosed single capillary flow cell devices, multiple capillary flow cell devices, capillary flow cell cartridges, and/or microfluidic devices and cartridges described herein. Additional description of the disclosed flow cell devices and cartridges may be found in PCT Patent Application Publication WO 2020/118255, which is incorporated herein by reference in its entirety.

In some instances, the systems disclosed herein may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 single capillary flow cell devices, multiple capillary flow cell devices, capillary flow cell cartridges, and/or microfluidic devices and cartridges. In some instances, the single capillary flow cell devices, multiple capillary flow cell devices, and/or microfluidic devices and cartridges may be fixed components of the disclosed systems. In some instances, the single capillary flow cell devices, multiple capillary flow cell devices, and/or microfluidic devices and cartridges may be removable, exchangeable components of the disclosed systems. In some instances, the single capillary flow cell devices, multiple capillary flow cell devices, and/or microfluidic devices and cartridges may be disposable or consumable components of the disclosed systems.

In some implementations, the disclosed single capillary flow cell devices (or single capillary flow cell cartridges) comprise a single capillary, e.g., a glass or fused-silica capillary, the lumen of which forms a fluid flow path through which reagents or solutions may flow, and the interior surface of which may form a sample support structure to which samples of interest are bound or tethered. In some implementations, the multi-capillary capillary flow cell devices (or multi-capillary flow cell cartridges) disclosed herein may comprise 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more than 20 capillaries configured for performing an analysis technique that further comprises imaging as a detection method.

In some instances, one or more capillaries may be packaged within a chassis to form a cartridge that facilitates ease-of-handling, incorporates adapters or connectors for making external fluid connections, and may optionally include additional integrated functionality such as reagent reservoirs, waste reservoirs, valves (e.g., microvalves), pumps (e.g., micropumps), etc., or any combination thereof.

FIG. 29 illustrates one non-limiting example of a single glass capillary flow cell device that comprises two fluidic adaptors—one affixed to each end of the piece of glass capillary—that are designed to mate with standard OD fluidic tubing to provide for convenient, interchangeable fluid connections with an external fluid flow control system. The fluidic adaptors can be attached to the capillary using any of a variety of techniques known to those of skill in the art including, but not limited to, press fit, adhesive bonding, solvent bonding, laser welding, etc., or any combination thereof.

In general, the capillaries used in the disclosed capillary flow cell devices and capillary flow cell cartridges will have at least one internal, axially-aligned fluid flow channel (or “lumen”) that runs the full length of the capillary. In some instances, the capillary may have two, three, four, five, or more than five internal, axially-aligned fluid flow channels (or “lumen”).

A number specified cross-sectional geometries for suitable capillaries (or the lumen thereof) are consistent with the disclosure herein including, but not limited to, circular, elliptical, square, rectangular, triangular, rounded square, rounded rectangular, or rounded triangular cross-sectional

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geometries. In some instances, the capillary (or lumen thereof) may have any specified cross-sectional dimension or set of dimensions. For example, in some instances the largest cross-sectional dimension of the capillary lumen (e.g. the diameter if the lumen is circular in shape, or the diagonal if the lumen is square or rectangular in shape) may range from about 10 μm to about 10 mm. In some aspects, the largest cross-sectional dimension of the capillary lumen may be at least 10 μm , at least 25 μm , at least 50 μm , at least 75 μm , at least 100 μm , at least 200 μm , at least 300 μm , at least 400 μm , at least 500 μm , at least 600 μm , at least 700 μm , at least 800 μm , at least 900 μm , at least 1 mm, at least 2 mm, at least 3 mm, at least 4 mm, at least 5 mm, at least 6 mm, at least 7 mm, at least 8 mm, at least 9 mm, or at least 10 mm.

In some aspects, the largest cross-sectional dimension of the capillary lumen may be at most 10 mm, at most 9 mm, at most 8 mm, at most 7 mm, at most 6 mm, at most 5 mm, at most 4 mm, at most 3 mm, at most 2 mm, at most 1 mm, at most 900 μm , at most 800 μm , at most 700 μm , at most 600 μm , at most 500 μm , at most 400 μm , at most 300 μm , at most 200 μm , at most 100 μm , at most 75 μm , at most 50 μm , at most 25 μm , or at most 10 μm . Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the largest cross-sectional dimension of the capillary lumen may range from about 100 μm to about 500 μm . Those of skill in the art will recognize that the largest cross-sectional dimension of the capillary lumen may have any value within this range, e.g., about 124 μm .

In some instances, e.g., wherein the lumen of the one or more capillaries in a flow cell device or cartridge has a square or rectangular cross-section, the distance between a first interior surface (e.g., a top or upper surface) and a second interior surface (e.g., a bottom or lower surface) that defines the gap height or thickness of a fluid flow channel may range from about 10 μm to about 500 μm . In some instances, the gap height may be at least 10 μm , at least 20 μm , at least 30 μm , at least 40 μm , at least 50 μm , at least 60 μm , at least 70 μm , at least 80 μm , at least 90 μm , at least 100 μm , at least 125 μm , at least 150 μm , at least 175 μm , at least 200 μm , at least 225 μm , at least 250 μm , at least 275 μm , at least 300 μm , at least 325 μm , at least 350 μm , at least 375 μm , at least 400 μm , at least 425 μm , at least 450 μm , at least 475 μm , or at least 500 μm . In some instances, the gap height may be at most 500 μm , at most 475 μm , at most 450 μm , at most 425 μm , at most 400 μm , at most 375 μm , at most 350 μm , at most 325 μm , at most 300 μm , at most 275 μm , at most 250 μm , at most 225 μm , at most 200 μm , at most 175 μm , at most 150 μm , at most 125 μm , at most 100 μm , at most 90 μm , at most 80 μm , at most 70 μm , at most 60 μm , at most 50 μm , at most 40 μm , at most 30 μm , at most 20 μm , or most 10 μm . Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the gap height may range from about 40 μm to about 125 μm . Those of skill in the art will recognize that the gap height may have any value within the range of values in this paragraph, e.g., about 122 μm .

In some instances, the length of the one or more capillaries used to fabricate the disclosed capillary flow cell devices or flow cell cartridges may range from about 5 mm to about 5 cm or greater. In some instances, the length of the one or more capillaries may be less than 5 mm, at least 5 mm, at least 1 cm, at least 1.5 cm, at least 2 cm, at least 2.5 cm, at least 3 cm, at least 3.5 cm, at least 4 cm, at least 4.5 cm, or at least 5 cm. In some instances, the length of the one or more capillaries may be at most 5 cm, at most 4.5 cm, at

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most 4 cm, at most 3.5 cm, at most 3 cm, at most 2.5 cm, at most 2 cm, at most 1.5 cm, at most 1 cm, or at most 5 mm. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the length of the one or more capillaries may range from about 1.5 cm to about 2.5 cm. Those of skill in the art will recognize that the length of the one or more capillaries may have any value within this range, e.g., about 1.85 cm. In some instances, devices or cartridges may comprise a plurality of two or more capillaries that are the same length. In some instances, devices or cartridges may comprise a plurality of two or more capillaries that are of different lengths.

The capillaries used for constructing the disclosed capillary flow cell devices or capillary flow cell cartridges may be fabricated from any of a variety of materials known to those of skill in the art including, but not limited to, glass (e.g., borosilicate glass, soda lime glass, etc.), fused silica (quartz), polymer (e.g., polystyrene (PS), macroporous polystyrene (MPPS), polymethylmethacrylate (PMMA), polycarbonate (PC), polypropylene (PP), polyethylene (PE), high density polyethylene (HDPE), cyclic olefin polymers (COP), cyclic olefin copolymers (COC), polyethylene terephthalate (PET), polydimethylsiloxane (PDMS), etc.), polyetherimide (PEI) and perfluoroelastomer (FFKM) as more chemically inert alternatives, or any combination thereof. PEI is somewhere between polycarbonate and PEEK in terms of both cost and chemical compatibility. FFKM is also known as Kalrez.

The one or more materials used to fabricate the capillaries are often optically transparent to facilitate use with spectroscopic or imaging-based detection techniques. In some instances, the entire capillary will be optically transparent. Alternately, in some instances, only a portion of the capillary (e.g., an optically transparent “window”) will be optically transparent.

The capillaries used for constructing the disclosed capillary flow cell devices and capillary flow cell cartridges may be fabricated using any of a variety of techniques known to those of skill in the art, where the choice of fabrication technique is often dependent on the choice of material used, and vice versa. Examples of suitable capillary fabrication techniques include, but are not limited to, extrusion, drawing, precision computer numerical control (CNC) machining and boring, laser photoablation, and the like.

In some implementations, the capillaries used in the disclosed capillary flow cell devices and cartridges may be off-the-shelf commercial products. Examples of commercial vendors that provide precision capillary tubing include Accu-Glass (St. Louis, Mo.; precision glass capillary tubing), Polymicro Technologies (Phoenix, Ariz.; precision glass and fused-silica capillary tubing), Friedrich & Dimmock, Inc. (Millville, N.J.; custom precision glass capillary tubing), and Drummond Scientific (Broomall, Pa.; OEM glass and plastic capillary tubing).

The fluidic adapters that are attached to the capillaries of the capillary flow cell devices and cartridges disclosed herein, and other components of the capillary flow cell devices or cartridges, may be fabricated using any of a variety of suitable techniques (e.g., extrusion molding, injection molding, compression molding, precision CNC machining, etc.) and materials (e.g., glass, fused-silica, ceramic, metal, polydimethylsiloxane, polystyrene (PS), macroporous polystyrene (MPPS), polymethylmethacrylate (PMMA), polycarbonate (PC), polypropylene (PP), polyethylene (PE), high density polyethylene (HDPE), cyclic olefin polymers (COP), cyclic olefin copolymers (COC), polyeth-

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ylene terephthalate (PET), etc.), where again the choice of fabrication technique is often dependent on the choice of material used, and vice versa.

FIG. 30 provides a non-limiting example of capillary flow cell cartridge that comprises two glass capillaries, fluidic adaptors (two per capillary in this example), and a cartridge chassis that mates with the capillaries and/or fluidic adapters such that the capillaries are held in a fixed orientation relative to the cartridge. In some instances, the fluidic adaptors may be integrated with the cartridge chassis. In some instances, the cartridge may comprise additional adapters that mate with the capillaries and/or capillary fluidic adapters. As noted elsewhere herein, in some instances, the cartridge may comprise additional functional components. In some instances, the capillaries are permanently mounted in the cartridge. In some instances, the cartridge chassis is designed to allow one or more capillaries of the flow cell cartridge to be interchangeable removed and replaced. For example, in some instances, the cartridge chassis may comprise a hinged “clamshell” configuration which allows it to be opened so that one or more capillaries may be removed and replaced. In some instances, the cartridge chassis is configured to mount on, for example, the stage of a fluorescence microscope or within a cartridge holder of a fluorescence imaging module or instrument system of the present disclosure.

In some instances, the disclosed flow cell devices may comprise microfluidic devices (or “microfluidic chips”) and cartridges, where the microfluidic devices are fabricated by forming fluid channels in one or more layers of a suitable material and comprise one or more fluid channels (e.g., “analysis” channels) configured for performing an analysis technique that further comprises imaging as a detection method. In some implementations, the microfluidic devices or cartridges disclosed herein may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more than 20 fluid channels (e.g., “analysis” fluid channels) configured for performing an analysis technique that further comprises imaging as a detection method. In some instances, the disclosed microfluidic devices may further comprise additional fluid channels (e.g., for dilution or mixing of reagents), reagent reservoirs, waste reservoirs, adapters for making external fluid connections, and the like, to provide integrated “lab-on-a-chip” functionality within the device.

A non-limiting example of microfluidic flow cell cartridge comprises a chip having two or more parallel glass channels formed on the chip, fluidic adaptors coupled to the chip, and a cartridge chassis that mates with the chip and/or fluidic adapters such that the chip is posited in a fixed orientation relative to the cartridge. In some instances, the fluidic adaptors may be integrated with the cartridge chassis. In some instances, the cartridge may comprise additional adapters that mate with the chip and/or fluidic adapters. In some instances, the chip is permanently mounted in the cartridge. In some instances, the cartridge chassis is designed to allow one or more chips of the flow cell cartridge to be interchangeably removed and replaced. For example, in some instances, the cartridge chassis may comprise a hinged “clamshell” configuration which allows it to be opened so that one or more chips may be removed and replaced. In some instances, the cartridge chassis is configured to mount on, for example, the stage of a microscope system or within a cartridge holder of an imaging system. Even though only one chip is described in the non-limiting example, it is understood that more than one chip can be used in the microfluidic flow cell cartridge. The flow cell cartridges of the present disclosure may comprise a single

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microfluidic chip or a plurality of microfluidic chips. In some instances, the flow cell cartridges of the present disclosure may comprise 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more than 20 microfluidic chips. The packaging of one or more microfluidic devices within a cartridge may facilitate ease-of-handling and correct positioning of the device within the optical imaging system.

The fluid channels within the disclosed microfluidic devices and cartridges may have an of a variety of cross-sectional geometries including, but not limited to, circular, elliptical, square, rectangular, triangular, rounded square, rounded rectangular, or rounded triangular cross-sectional geometries. In some instances, the fluid channels may have any specified cross-sectional dimension or set of dimensions. For example, in some instances, the height (e.g., gap height), width, or largest cross-sectional dimension of the fluid channels (e.g., the diagonal if the fluid channel has a square, rounded square, rectangular, or rounded rectangular cross-section) may range from about 10 μm to about 10 mm. In some aspects, the height (e.g., gap height), width, or largest cross-sectional dimension of the fluid channels may be at least 10 μm , at least 25 μm , at least 50 μm , at least 75 μm , at least 100 μm , at least 200 μm , at least 300 μm , at least 400 μm , at least 500 μm , at least 600 μm , at least 700 μm , at least 800 μm , at least 900 μm , at least 1 mm, at least 2 mm, at least 3 mm, at least 4 mm, at least 5 mm, at least 6 mm, at least 7 mm, at least 8 mm, at least 9 mm, or at least 10 mm. In some aspects, the height (e.g., gap height), width, or largest cross-sectional dimension of the fluid channels may be at most 10 mm, at most 9 mm, at most 8 mm, at most 7 mm, at most 6 mm, at most 5 mm, at most 4 mm, at most 3 mm, at most 2 mm, at most 1 mm, at most 900 μm , at most 800 μm , at most 700 μm , at most 600 μm , at most 500 μm , at most 400 μm , at most 300 μm , at most 200 μm , at most 100 μm , at most 75 μm , at most 50 μm , at most 25 μm , or at most 10 μm . Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the height (e.g., gap height), width, or largest cross-sectional dimension of the fluid channels may range from about 20 μm to about 200 μm . Those of skill in the art will recognize that the height (e.g., gap height), width, or largest cross-sectional dimension of the fluid channels may have any value within this range, e.g., about 122 μm .

In some instances, the length of the fluid channels in the disclosed microfluidic devices and cartridges may range from about 5 mm to about 10 cm or greater. In some instances, the length of the fluid channels may be less than 5 mm, at least 5 mm, at least 1 cm, at least 1.5 cm, at least 2 cm, at least 2.5 cm, at least 3 cm, at least 3.5 cm, at least 4 cm, at least 4.5 cm, at least 5 cm, at least 6 cm, at least 7 cm, at least 8 cm, at least 9 cm, or at least 10 cm. In some instances, the length of the fluid channels may be at most 10 cm, at most 9 cm, at most 8 cm, at most 7 cm, at most 6 cm, at most 5 cm, at most 4.5 cm, at most 4 cm, at most 3.5 cm, at most 3 cm, at most 2.5 cm, at most 2 cm, at most 1.5 cm, at most 1 cm, or at most 5 mm. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the length of the fluid channels may range from about 1.5 cm to about 2.5 cm. Those of skill in the art will recognize that the length of the fluid channels may have any value within this range, e.g., about 1.35 cm. In some instances, the microfluidic devices or cartridges may comprise a plurality of fluid channels that are the same length.

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In some instances, the microfluidic devices or cartridges may comprise a plurality of fluid channels that are of different lengths.

The disclosed microfluidic devices will comprise at least one layer of material having one or more fluid channels formed therein. In some instances, the microfluidic chip may include two layers bonded together to form one or more fluid channels. In some instances, the microfluidic chip may include three or more layers bonded together to form one or more fluid channels. In some instances, the microfluidic fluid channels may have an open top. In some instances, the microfluidic fluid channels may be fabricated within one layer, e.g., the top surface of a bottom layer, and sealed by bonding the top surface of the bottom layer to the bottom surface of a top layer of material. In some instances, the microfluidic channels may be fabricated within one layer, e.g., as patterned channels the depth of which extends through the full thickness of the layer, which is then sandwiched between and bonded to two non-patterned layers to seal the fluid channels. In some instances, the microfluidic channels are fabricated by the removal of a sacrificial layer on the surface of a substrate. This method does not require the bulk substrate (e.g., a glass or silicon wafer) to be etched away. Instead, the fluid channels are located on the surface of the substrate. In some instances, the microfluidic channels may be fabricated in or on the surface of a substrate and then sealed by deposition of a conformal film or layer on the surface of the substrate to create sub-surface or buried fluid channels in the chip.

The microfluidic chips can be manufactured using a combination of microfabrication processes. Because the devices are microfabricated, substrate materials will typically be selected based upon their compatibility with known microfabrication techniques, e.g., photolithography, wet chemical etching, laser ablation, laser irradiation, air abrasion techniques, injection molding, embossing, and other techniques. The substrate materials are also generally selected for their compatibility with the full range of conditions to which the microfluidic devices may be exposed, including extremes of pH, temperature, salt concentration, and application of electromagnetic (e.g. light) or electric fields.

The disclosed microfluidic chips may be fabricated from any of a variety of materials known to those of skill in the art including, but not limited to, glass (e.g., borosilicate glass, soda lime glass, etc.), fused-silica (quartz), silicon, a polymer (e.g., polystyrene (PS), macroporous polystyrene (MPPS), polymethylmethacrylate (PMMA), polycarbonate (PC), polypropylene (PP), polyethylene (PE), high density polyethylene (HDPE), cyclic olefin polymers (COP), cyclic olefin copolymers (COC), polyethylene terephthalate (PET), polydimethylsiloxane (PDMS), etc.), polyetherimide (PEI) and perfluoroelastomer (FFKM) (as more chemically inert alternatives), or any combination thereof. In some preferred instances, the substrate material(s) may include silica-based substrates, such as borosilicate glass, and quartz, as well as other substrate materials.

The disclosed microfluidic devices may be fabricated using any of a variety of techniques known to those of skill in the art, where the choice of fabrication technique is often dependent on the choice of material used, and vice versa. The microfluidic channels on the chip can be constructed using techniques suitable for forming micro-structures or micro-patterns on the surface of a substrate. In some instances, the fluid channels are formed by laser irradiation. In some instances, the microfluidic channels are formed by focused femtosecond laser radiation. In some instances, the

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microfluidic channels are formed by photolithography and etching including, but not limited to, chemical etching, plasma etching, or deep reactive ion etching. In some instances, the microfluidic channels are formed using laser etching. In some instances, the microfluidic channels are formed using a direct-write lithography technique. Examples of direct-write lithography include electron beam direct-write and focused ion beam milling.

In additional preferred instances, the substrate material(s) may comprise polymeric materials, e.g., plastics, such as polymethylmethacrylate (PMMA), polycarbonate, polytetrafluoroethylene (TEFLON™), polyvinylchloride (PVC), polydimethylsiloxane (PDMS), polysulfone, and the like. Such polymeric substrates may be readily patterned or micromachined using available microfabrication techniques, such as those described above. In some instances, microfluidic chips may be fabricated from polymeric materials, e.g., from microfabricated masters, using well known molding techniques, such as injection molding, embossing, stamping, or by polymerizing the polymeric precursor material within a mold (see, e.g., U.S. Pat. No. 5,512,131). In some instances, such polymeric substrate materials are preferred for their ease of manufacture, low cost, and disposability, as well as their general inertness to most extreme reaction conditions. As with flow cell devices fabricated from other materials, e.g., glass, flow cell devices fabricated from these polymeric materials may include treated surfaces, e.g., derivatized or coated surfaces, to enhance their utility in the microfluidic system, as will be discussed in more detail below.

The fluid channels and/or fluid chambers of the microfluidic devices are typically fabricated into the upper surface of a first substrate as microscale channels (e.g., grooves, indentations, etc.) using the above described microfabrication techniques. The first substrate comprises a top side having a first planar surface and a bottom side. In the microfluidic devices prepared in accordance with the methods described herein, the plurality of fluid channels (e.g., grooves and/or indentations) are formed on the first planar surface. In some instances, the fluid channels (e.g., grooves and/or indentations) formed in the first planar surface (prior to bonding to a second substrate) have a bottom and side walls, with the top remaining open. In some instances, the fluid channels (e.g., grooves and/or indentations) formed in the first planar surface (prior to bonding to a second substrate) have a bottom and side walls and the top remaining closed. In some instances, the fluid channels (e.g., grooves and/or indentations) formed in the first planar surfaces (prior to bonding to a second substrate) have only side walls and no top or bottom surface (i.e., the fluid channels span the full thickness of the first substrate).

Fluid channels and chambers may be sealed by placing the first planar surface of the first substrate in contact with, and bonding to, the planar surface of a second substrate to form the channels and/or chambers (e.g., the interior portion) of the device at the interface of these two components. In some instances, after the first substrate is bonded to a second substrate, the structure may further be placed in contact with and bonded to a third substrate. In some instances, the third substrate may be placed in contact with the side of the first substrate that is not in contact with the second substrate. In some instances, the first substrate is placed between the second substrate and the third substrate. In some instances, the second substrate and the third substrate can cover and/or seal the grooves, indentations, or apertures formed on the first substrate to form the channels

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and/or chambers (e.g., the interior portion) of the device at the interface of these components.

The device can have openings that are oriented such that they are in fluid communication with at least one of the fluid channels and/or fluid chambers formed in the interior portion of the device, thereby forming fluid inlets and/or fluid outlets. In some instances, the openings are formed on the first substrate. In some instances, the openings are formed on the first and the second substrate. In some instances, the openings are formed on the first, the second, and the third substrate. In some instances, the openings are positioned at the top side of the device. In some instances, the openings are positioned at the bottom side of the device. In some instances, the openings are positioned at the first and/or the second ends of the device, and the channels run along the direction from the first end to the second end.

Conditions under which substrates may be bonded together are generally widely understood by those of skill in the art, and such bonding of substrates is generally carried out by any of a variety of methods, the choice of which may vary depending upon the nature of the substrate materials used. For example, thermal bonding of substrates may be applied to a number of substrate materials including, e.g., glass or silica-based substrates, as well as some polymer based-substrates. Such thermal bonding techniques typically comprise mating the substrate surfaces that are to be bonded under conditions of elevated temperature and, in some cases, application of external pressure. The precise temperatures and pressures utilized will generally vary depending upon the nature of the substrate materials used.

For example, for silica-based substrate materials, i.e., glass (borosilicate glass, Pyrex™ soda lime glass, etc.), fused-silica (quartz), and the like, thermal bonding of substrates is typically carried out at temperatures ranging from about 500° C. to about 1400° C., and preferably, from about 500° C. to about 1200° C. For example, soda lime glass is typically bonded at temperatures of around 550° C., whereas borosilicate glass is typically thermally bonded at or near 800° C. Quartz substrates, on the other hand, are typically thermally bonded at temperatures at or near 1200° C. These bonding temperatures are typically achieved by placing the substrates to be bonded into high temperature annealing ovens.

Polymeric substrates that are thermally bonded, on the other hand, will typically utilize lower temperatures and/or pressures than silica-based substrates, in order to prevent excessive melting of the substrates and/or distortion, e.g., flattening of the interior portion of the device (i.e., the fluid channels or chambers). Generally, such elevated temperatures for bonding polymeric substrates will vary from about 80° C. to about 200° C., depending upon the polymeric material used, and will preferably be between about 90° C. and about 150° C. Because of the significantly reduced temperatures required for bonding polymeric substrates, such bonding may typically be carried out without the need for the high temperature ovens used in the bonding of silica-based substrates. This allows incorporation of a heat source within a single integrated bonding system, as described in greater detail below.

Adhesives may also be used to bond substrates together according to well-known methods, which typically comprise applying a layer of adhesive between the substrates that are to be bonded and pressing them together until the adhesive sets. A variety of adhesives may be used in accordance with these methods, including, e.g., UV curable adhesives, that are commercially available. Alternative methods may also be used to bond substrates together in accordance with the

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present invention, including e.g., acoustic or ultrasonic welding and/or solvent welding of polymeric parts.

Typically, a number of the described microfluidic chips or devices will be manufactured at the same time, e.g., using “wafer-scale” fabrication. For example, polymeric sub-
strates may be stamped or molded in large separable sheets which can then be mated and bonded together. Individual devices or bonded substrates may then be separated from the larger sheet by cutting or dicing. Similarly, for silica-based substrates, individual devices can be fabricated from larger substrate wafers or plates, allowing higher throughput of the manufacturing process. Specifically, a plurality of fluid channel structures can be fabricated on a first substrate wafer or plate, which is then overlaid with and bonded to a second substrate wafer or plate, and optionally further overlaid with and bonded to a third substrate wafer or plate. The individual devices are then segmented from the larger substrates using known methods, such as sawing, scribing and breaking, and the like.

As noted above, the top or second substrate is overlaid upon the bottom or first substrate to seal the various channels and chambers. In carrying out the bonding process according to the methods of the present disclosure, the bonding of the first and second substrates may be carried out using vacuum and/or pressure to maintain the two substrate surfaces in optimal contact. In particular, the bottom substrate may be maintained in optimal contact with the top substrate by, e.g., mating the planar surface of the bottom substrate with the planar surface of the top substrate and applying a vacuum through holes that are disposed through the top substrate. Typically, application of a vacuum to holes in the top substrate is carried out by placing the top substrate on a vacuum chuck, which typically comprises a mounting table or surface, having an integrated vacuum source. In the case of silica-based substrates, the bonded substrates are subjected to elevated temperatures in order to create an initial bond, so that the bonded substrates may then be transferred to the annealing oven, without any shifting relative to each other.

Alternate bonding systems for incorporation with the apparatus described herein include, e.g., adhesive dispensing systems, for applying adhesive layers between the two planar surfaces of the substrates. This may be done by applying the adhesive layer prior to mating the substrates, or by placing an amount of the adhesive at one edge of the adjoining substrates and allowing the wicking action of the two mated substrates to draw the adhesive across the space between the two substrates.

In certain instances, the overall bonding system can include automatable systems for placing the top and bottom substrates on the mounting surface and aligning them for subsequent bonding. Typically, such systems include translation systems for moving either the mounting surface or one or more of the top and bottom substrates relative to each other. For example, robotic systems may be used to lift, translate and place each of the top and bottom substrates upon the mounting table, and within the alignment structures, in turn. Following the bonding process, such systems also can remove the finished product from the mounting surface and transfer these mated substrates to a subsequent operation, e.g., a separation or dicing operation, an annealing oven for silica-based substrates, etc., prior to placing additional substrates thereon for bonding.

In some instances, the manufacturing of the microfluidic chip includes the layering or laminating of two or more layers of substrate, e.g., patterned and non-patterned polymeric sheets, in order to produce the chip. For example, in

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microfluidic devices, the microfluidic features of the device are typically produced by laser irradiation, etching, or otherwise fabricating features into the surface of a first layer. A second layer is then laminated or bonded to the surface of the first to seal these features and provide the fluidic elements of the device, e.g., the fluid channels.

As noted above, in some instances one or more capillary flow cell devices or microfluidic chips may be mounted in a cartridge chassis to form a capillary flow cell cartridge or microfluidic cartridge. In some instances, the capillary flow cell cartridge or microfluidic cartridge may further comprise additional components that are integrated with the cartridge to provide enhanced performance for specific applications. Examples of additional components that may be integrated into the cartridge include, but are not limited to, adapters or connectors for making fluidic connections to other components of the system, fluid flow control components (e.g., miniature valves, miniature pumps, mixing manifolds, etc.), temperature control components (e.g., resistive heating elements, metal plates that serve as heat sources or sinks, piezoelectric (Peltier) devices for heating or cooling, temperature sensors), or optical components (e.g., optical lenses, windows, filters, mirrors, prisms, fiber optics, and/or light-emitting diodes (LEDs) or other miniature light sources that may collectively be used to facilitate spectroscopic measurements and/or imaging of one or more capillary or fluid flow channels.

The fluidic adaptors, cartridge chassis, and other cartridge components may be attached to the capillaries, capillary flow cell device(s), microfluidic chip(s) (or fluid channels within the chip) using any of a variety of techniques known to those of skill in the art including, but not limited to, press fit, adhesive bonding, solvent bonding, laser welding, etc., or any combination thereof. In some instances, the inlet(s) and/or outlet(s) of the microfluidic channels in the microfluidic chip are apertures on the top surface of the chip, and the fluidic adaptors can be attached or coupled to the inlet(s) and/or outlet(s) of the microfluidic channels within the chip. In some instances, the cartridge may comprise additional adapters (i.e., in addition to the fluidic adapters) that mate with the chip and/or fluidic adapters and help to position the chip within the cartridge. These adapters may be constructed using the same fabrication techniques and materials as those outlined above for the fluidic adapters.

The cartridge chassis (or “housing”) may be fabricated from metal and/or polymer materials such as aluminum, anodized aluminum, polycarbonate (PC), acrylic (PMMA), or Ultem (PEI), while other materials are also consistent with the present disclosure. A housing may be fabricated using CNC machining and/or molding techniques, and designed so that one, two, or more than two capillaries or microfluidic chips are constrained by the chassis in a fixed orientation to create one or more independent flow channels. The capillaries or chips may be mounted in the chassis using, e.g., a compression fit design, or by mating with compressible adapters made of silicone or a fluoroelastomer. In some instances, two or more components of the cartridge chassis (e.g., an upper half and a lower half) are assembled using, e.g., screws, clips, clamps, or other fasteners so that the two halves are separable. In some instances, two or more components of the cartridge chassis are assembled using, e.g., adhesives, solvent bonding, or laser welding so that the two or more components are permanently attached.

Flow Cell Surface Coatings:

In some instances, one or more interior surfaces of the capillary lumens or microfluidic channels in the disclosed flow cell devices may be coated using any of a variety of

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surface modification techniques or polymer coatings known to those of skill in the art. In some instances, the coatings may be formulated to increase or maximize the number of available binding sites (e.g., tethered oligonucleotide adapter/primer sequences) on the one or more interior surfaces to increase or maximize a foreground signal, e.g., a fluorescence signal arising from labeled nucleic acid molecules hybridized to tethered oligonucleotide adapter/primer sequences. In some instances, the coatings may be formulated to decrease or minimize nonspecific binding of fluorophores and other small molecules, or labeled or unlabeled nucleotides, proteins, enzymes, antibodies, oligonucleotides, or nucleic acid molecules (e.g., DNA, RNA, etc.), in order to decrease or minimize a background signal, e.g., background fluorescence arising from the nonspecific binding of labeled biomolecules or from autofluorescence of a sample support structure. The combination of increased foreground signal and reduced background signal that may be achieved in some instances through the use of the disclosed coatings may thus provide improved signal-to-noise ratio (SNR) in spectroscopic measurements or improved contrast-to-noise ratio (CNR) in imaging methods.

As will be discussed in more detail below, the disclosed hydrophilic, polymer-coated flow cell devices, optionally used in combination with the improved hybridization and/or amplification protocols, yield solid-phase bioassay reactions that exhibit: (i) negligible non-specific binding of protein and other reaction components (thus reducing or minimizing substrate background), (ii) negligible non-specific nucleic acid amplification product, and (iii) provide tunable nucleic acid amplification reactions. Although described herein primarily in the context of nucleic acid hybridization, amplification, and sequencing assays, it will be understood by those of skill in the art that the disclosed low-binding supports may be used in any of a variety of other bioassay formats including, but not limited to, sandwich immunoassays, enzyme-linked immunosorbent assays (ELISAs), etc.

In a preferred aspect, one or more layers of a coating material may be applied to the interior flow cell device surfaces, where the number of layers and/or the material composition of each layer is chosen to adjust one or more surface properties of the interior flow cell device surfaces, as noted in U.S. patent application Ser. No. 16/363,842. Examples of surface properties that may be adjusted include, but are not limited to, surface hydrophilicity/hydrophobicity, overall coating thickness, the surface density of chemically-reactive functional groups, the surface density of grafted linker molecules or oligonucleotide adapters/primers, etc. In some preferred applications, one or more surface properties of the capillary or channel lumen are adjusted to, for example, (i) provide for very low non-specific binding of proteins, oligonucleotides, fluorophores, and other molecular components of chemical or biological analysis applications, including solid-phase nucleic acid amplification and/or sequencing applications, (ii) provide for improved solid-phase nucleic acid hybridization specificity and efficiency, and (iii) provide for improved solid-phase nucleic acid amplification rate, specificity, and efficiency.

Any of a variety of molecules known to those of skill in the art including, but not limited to, silanes, amino acids, peptides, nucleotides, oligonucleotides, other monomers or polymers, or combinations thereof may be used in creating the one or more chemically-modified layers on the interior flow cell device surfaces, where the choice of components used may be varied to alter one or more properties of the support surface, e.g., the surface density of functional groups and/or tethered oligonucleotide primers, the hydro-

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philicity/hydrophobicity of the support surface, or the three three-dimensional nature (i.e., "thickness") of the support surface.

The attachment chemistry used to graft a first chemically-modified layer to an interior surface of the flow cell (capillary or channel) will generally be dependent on both the material from which the flow cell device is fabricated and the chemical nature of the layer. In some instances, the first layer may be covalently attached to the interior flow cell device surfaces. In some instances, the first layer may be non-covalently attached, e.g., adsorbed to the surface through non-covalent interactions such as electrostatic interactions, hydrogen bonding, or van der Waals interactions between the surface and the molecular components of the first layer. In either case, the substrate surface may be treated prior to attachment or deposition of the first layer. Any of a variety of surface preparation techniques known to those of skill in the art may be used to clean or treat the support surface. For example, glass or silicon surfaces may be acid-washed using a Piranha solution (a mixture of sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2)) and/or cleaned using an oxygen plasma treatment method.

Silane chemistries constitute one non-limiting approach for covalently modifying the silanol groups on glass or silicon surfaces to attach more reactive functional groups (e.g., amines or carboxyl groups), which may then be used in coupling linker molecules (e.g., linear hydrocarbon molecules of various lengths, such as C6, C12, C18 hydrocarbons, or linear polyethylene glycol (PEG) molecules) or layer molecules (e.g., branched PEG molecules or other polymers) to the surface. Examples of suitable silanes that may be used in creating any of the disclosed low binding support surfaces include, but are not limited to, (3-Aminopropyl)trimethoxysilane (APTMS), (3-Aminopropyl)triethoxysilane (APTES), any of a variety of PEG-silanes (e.g., comprising molecular weights of 1K, 2K, 5K, 10K, 20K, etc.), amino-PEG silane (i.e., comprising a free amino functional group), maleimide-PEG silane, biotin-PEG silane, and the like.

Examples of preferred polymers that may be used to create one or more layers of low non-specific binding material in any of the disclosed support surfaces include, but are not limited to, polyethylene glycol (PEG) of various molecular weights and branching structures, streptavidin, polyacrylamide, polyester, dextran, poly-lysine, and poly-lysine copolymers, or any combination thereof. Examples of conjugation chemistries that may be used to graft one or more layers of material (e.g. polymer layers) to the support surface and/or to cross-link the layers to each other include, but are not limited to, biotin-streptavidin interactions (or variations thereof), His tag-Ni/NTA conjugation chemistries, methoxy ether conjugation chemistries, carboxylate conjugation chemistries, amine conjugation chemistries, NHS esters, maleimides, thiol, epoxy, azide, hydrazide, alkyne, isocyanate, and silane.

In some instances, the number of layers of polymer or other chemical layers on the interior flow cell device surfaces may range from 1 to about 10, or greater than 10. In some instances, the number of layers is at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10. In some instances, the number of layers may be at most 10, at most 9, at most 8, at most 7, at most 6, at most 5, at most 4, at most 3, at most 2, or at most 1. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the number of layers may range from about 2 to about 4. In some

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instances, the one or more layers may all comprise the same material. In some instances, each layer may comprise a different material. In some instances, a plurality of layers may comprise a plurality of materials.

One or more layers of a multi-layered surface may comprise a branched polymer or may be linear. Examples of suitable branched polymers include, but are not limited to, branched PEG, branched poly(vinyl alcohol) (branched PVA), branched poly(vinyl pyridine), branched poly(vinyl pyrrolidone) (branched PVP), branched poly(acrylic acid) (branched PAA), branched polyacrylamide, branched poly(N-isopropylacrylamide) (branched PNIPAM), branched poly(methyl methacrylate) (branched PMA), branched poly(2-hydroxyethyl methacrylate) (branched PHEMA), branched poly(oligo(ethylene glycol) methyl ether methacrylate) (branched POEGMA), branched polyglutamic acid (branched PGA), branched poly-lysine, branched poly-glucoside, and dextran.

In some instances, the branched polymers used to create one or more layers of any of the multi-layered surfaces disclosed herein may comprise at least 4 branches, at least 5 branches, at least 6 branches, at least 7 branches, at least 8 branches, at least 9 branches, at least 10 branches, at least 12 branches, at least 14 branches, at least 16 branches, at least 18 branches, at least 20 branches, at least 22 branches, at least 24 branches, at least 26 branches, at least 28 branches, at least 30 branches, at least 32 branches, at least 34 branches, at least 36 branches, at least 38 branches, or at least 40 branches. Molecules often exhibit a ‘power of 2’ number of branches, such as 2, 4, 8, 16, 32, 64, or 128 branches.

In some instances, the resulting functional end groups distal from the surface following the deposition of one or more layers, e.g., polymer layers can include, but are not limited to, biotin, methoxy ether, carboxylate, amine, NHS ester, maleimide, and bis-silane.

Linear, branched, or multi-branched polymers used to create one or more layers of any of the multi-layered surfaces disclosed herein may have a molecular weight of at least 500 Daltons, at least 1,000 Daltons, at least 1,500 Daltons, at least 2,000 Daltons, at least 2,500 Daltons, at least 3,000 Daltons, at least 3,500 Daltons, at least 4,000 Daltons, at least 4,500 Daltons, at least 5,000 Daltons, at least 7,500 Daltons, at least 10,000 Daltons, at least 12,500 Daltons, at least 15,000 Daltons, at least 17,500 Daltons, at least 20,000 Daltons, at least 25,000 Daltons, at least 30,000 Daltons, at least 35,000 Daltons, at least 40,000 Daltons, at least 45,000 Daltons, or at least 50,000 Daltons. In some instances, the linear, branched, or multi-branched polymers used to create one or more layers of any of the multi-layered surfaces disclosed herein may have a molecular weight of at most 50,000 Daltons, at most 45,000 Daltons, at most 40,000 Daltons, at most 35,000 Daltons, at most 30,000 Daltons, at most 25,000 Daltons, at most 20,000 Daltons, at most 17,500 Daltons, at most 15,000 Daltons, at most 12,500 Daltons, at most 10,000 Daltons, at most 7,500 Daltons, at most 5,000 Daltons, at most 4,500 Daltons, at most 4,000 Daltons, at most 3,500 Daltons, at most 3,000 Daltons, at most 2,500 Daltons, at most 2,000 Daltons, at most 1,500 Daltons, at most 1,000 Daltons, or at most 500 Daltons. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the molecular weight of linear, branched, or multi-branched polymers used to create one or more layers of any of the multi-layered surfaces disclosed herein may range from about 1,500 Daltons to about 20,000 Daltons. Those of skill

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in the art will recognize that the molecular weight of linear, branched, or multi-branched polymers used to create one or more layers of any of the multi-layered surfaces disclosed herein may have any value within this range, e.g., about 1,260 Daltons.

In some instances, two or more layers may be covalently coupled to each other or internally cross-linked to improve the stability of the resulting surface. In some instances, e.g., wherein at least one layer of a multi-layered surface comprises a branched polymer, the number of covalent bonds between a branched polymer molecule of the layer being deposited and molecules of the previous layer may range from about one covalent linkage per molecule and about 32 covalent linkages per molecule. In some instances, the number of covalent bonds between a branched polymer molecule of the new layer and molecules of the previous layer may be at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 12, at least 14, at least 16, at least 18, at least 20, at least 22, at least 24, at least 26, at least 28, at least 30, or at least 32, or more than 32 covalent linkages per molecule. In some instances, the number of covalent bonds between a branched polymer molecule of the new layer and molecules of the previous layer may be at most 32, at most 30, at most 28, at most 26, at most 24, at most 22, at most 20, at most 18, at most 16, at most 14, at most 12, at most 10, at most 9, at most 8, at most 7, at most 6, at most 5, at most 4, at most 3, at most 2, or at most 1. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the number of covalent bonds between a branched polymer molecule of the new layer and molecules of the previous layer may range from about 4 to about 16. Those of skill in the art will recognize that the number of covalent bonds between a branched polymer molecule of the new layer and molecules of the previous layer may have any value within this range, e.g., about 11 in some instances, or an average number of about 4.6 in other instances.

Any reactive functional groups that remain following the coupling of a material layer to the interior flow cell device surfaces may optionally be blocked by coupling a small, inert molecule using a high yield coupling chemistry. For example, in the case that amine coupling chemistry is used to attach a new material layer to the previous one, any residual amine groups may subsequently be acetylated or deactivated by coupling with a small amino acid such as glycine.

In order to scale binding site surface density, e.g., oligonucleotide adapter/primer surface density, and add additional dimensionality to hydrophilic or amphoteric surfaces, substrates comprising multi-layer coatings of PEG and other hydrophilic polymers have been developed. By using hydrophilic and amphoteric surface layering approaches that include, but are not limited to, the polymer/co-polymer materials described below, it is possible to increase adapter/primer loading density on the surface significantly. Traditional PEG coating approaches use monolayer primer deposition, which has been tested and reported for single molecule sequencing applications but do not yield high copy numbers for nucleic acid amplification applications. As described herein, “layering” can be accomplished using traditional crosslinking approaches with any compatible polymer or monomer subunits such that a surface comprising two or more highly crosslinked layers can be built sequentially. Examples of suitable polymers include, but are not limited to, streptavidin, polyacrylamide, polyester, dextran, poly-lysine, and copolymers of poly-lysine and PEG.

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In some instances, the different layers may be cross-linked to each other through any of a variety of conjugation reactions including, but not limited to, biotin-streptavidin binding, azide-alkyne click reaction, amine-NETS ester reaction, thiol-maleimide reaction, and ionic interactions between positively charged polymer and negatively charged polymer. In some instances, high adapter/primer density materials may be constructed in solution and subsequently layered onto the surface in multiple steps.

Exemplary PEG multilayers include PEG (8 arm, 16 arm, 8 arm) on PEG-amine-APTES. Similar concentrations were observed for 3-layer multi-arm PEG (8 arm, 16 arm, 8 arm) and (8 arm, 64 arm, 8 arm) on PEG-amine-APTES exposed to 8 uM primer, and 3-layer multi-arm PEG (8 arm, 8 arm, 8 arm) using star-shape PEG-amine to replace 16 arm and 64 arm. PEG multilayers having comparable first, second and third PEG layers are also contemplated.

In some instances, the resultant surface density of binding sites on the interior flow cell device surfaces, e.g., oligonucleotide adapter/primer surface densities, may range from about 100 primer molecules per μm^2 to about 1,000,000 primer molecules per μm^2 . In some instances, the surface density of binding sites may be at least 100, at least 200, at least 300, at least 400, at least 500, at least 600, at least 700, at least 800, at least 900, at least 1,000, at least 1,500, at least 2,000, at least 2,500, at least 3,000, at least 3,500, at least 4,000, at least 4,500, at least 5,000, at least 5,500, at least 6,000, at least 6,500, at least 7,000, at least 7,500, at least 8,000, at least 8,500, at least 9,000, at least 9,500, at least 10,000, at least 15,000, at least 20,000, at least 25,000, at least 30,000, at least 35,000, at least 40,000, at least 45,000, at least 50,000, at least 55,000, at least 60,000, at least 65,000, at least 70,000, at least 75,000, at least 80,000, at least 85,000, at least 90,000, at least 95,000, at least 100,000, at least 150,000, at least 200,000, at least 250,000, at least 300,000, at least 350,000, at least 400,000, at least 450,000, at least 500,000, at least 550,000, at least 600,000, at least 650,000, at least 700,000, at least 750,000, at least 800,000, at least 850,000, at least 900,000, at least 950,000, or at least 1,000,000 molecules per μm^2 . In some instances, the surface density of binding sites may be at most 1,000,000, at most 950,000, at most 900,000, at most 850,000, at most 800,000, at most 750,000, at most 700,000, at most 650,000, at most 600,000, at most 550,000, at most 500,000, at most 450,000, at most 400,000, at most 350,000, at most 300,000, at most 250,000, at most 200,000, at most 150,000, at most 100,000, at most 95,000, at most 90,000, at most 85,000, at most 80,000, at most 75,000, at most 70,000, at most 65,000, at most 60,000, at most 55,000, at most 50,000, at most 45,000, at most 40,000, at most 35,000, at most 30,000, at most 25,000, at most 20,000, at most 15,000, at most 10,000, at most 9,500, at most 9,000, at most 8,500, at most 8,000, at most 7,500, at most 7,000, at most 6,500, at most 6,000, at most 5,500, at most 5,000, at most 4,500, at most 4,000, at most 3,500, at most 3,000, at most 2,500, at most 2,000, at most 1,500, at most 1,000, at most 900, at most 800, at most 700, at most 600, at most 500, at most 400, at most 300, at most 200, or at most 100 molecules per μm^2 . Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the surface density of binding sites may range from about 10,000 molecules per μm^2 to about 100,000 molecules per μm^2 . Those of skill in the art will recognize that the surface density of binding sites may have any value within this range, e.g., about 3,800 molecules per μm^2 in some instances, or about 455,000 molecules per μm^2 in other instances. In some instances, as

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will be discussed further below for nucleic acid sequencing applications, the surface density of template library nucleic acid sequences (e.g., sample DNA molecules) initially hybridized to adapter or primer sequences tethered to the interior flow cell device surfaces may be less than or equal to that indicated for the surface density of binding sites. In some instances, as will also be discussed further below, the surface density of clonally-amplified template library nucleic acid sequences hybridized to adapter or primer sequences on the interior flow cell device surfaces may span the same range or a different range as that indicated for the surface density of tethered oligonucleotide adapters or primers.

Local surface densities of binding sites on the interior flow cell device surfaces as listed above do not preclude variation in density across a surface, such that a surface may comprise a region having a binding site density of, for example, 500,000/ μm^2 , while also comprising at least a second region having a substantially different local surface density.

In some instances, capture probes, e.g., oligonucleotide primers with different base sequences and base modifications (or other biomolecules, e.g., enzymes or antibodies) may be tethered to one or more layers of the resulting surface at various surface densities. In some instances, for example, both surface functional group density and the capture probe concentration used for coupling may be varied to target a certain capture probe surface density range. Additionally, capture probe surface density may be controlled by diluting capture probes with other “inert” molecules that carry the same reactive functional group for coupling to the surface. For example, amine-labeled oligonucleotide probes can be diluted with amine-labeled polyethylene glycol in a reaction with an NETS-ester coated surface to reduce the final primer density. In the case of oligonucleotide adapters/primers, probe sequences with different lengths of linker between the hybridization region and the surface attachment functional group may also be applied to vary surface density. Example of suitable linkers include poly-T and poly-A strands at the 5' end of the primer (e.g., 0 to 20 bases), PEG linkers (e.g., 3 to 20 monomer units), and carbon-chain (e.g., C6, C12, C18, etc.). To measure or estimate the capture probe surface density, fluorescently labeled capture probes may be tethered to the surface and a fluorescence reading then compared with that for a calibration solution comprising a known concentration of the fluorophore.

In some instances, the degree of hydrophilicity (or “wettability” with aqueous solutions) of the disclosed support surfaces, e.g., interior flow cell device surfaces, may be assessed, for example, through the measurement of water contact angles in which a small droplet of water is placed on the surface and its angle of contact with the surface is measured using, e.g., an optical tensiometer. In some instances, a static contact angle may be determined. In some instances, an advancing or receding contact angle may be determined. In some instances, the water contact angle for the hydrophilic, low-binding support surfaced disclosed herein may range from about 0 degrees to about 50 degrees. In some instances, the water contact angle for the hydrophilic, low-binding support surfaced disclosed herein may no more than 50 degrees, 45 degrees, 40 degrees, 35 degrees, 30 degrees, 25 degrees, 20 degrees, 18 degrees, 16 degrees, 14 degrees, 12 degrees, 10 degrees, 8 degrees, 6 degrees, 4 degrees, 2 degrees, or 1 degree. In many cases the contact angle is no more than any value within this range, e.g., no more than 40 degrees. Those of skill in the art will realize

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that a given hydrophilic, low-binding support surface of the present disclosure may exhibit a water contact angle having a value of anywhere within this range, e.g., about 27 degrees. In some instances, the disclosed low nonspecific binding surfaces have a water contact angle of less than 45 degrees. In some instances, the disclosed low nonspecific binding surfaces have a water contact angle of less than 35 degrees.

As noted, the hydrophilic coated interior flow cell device surfaces of the present disclosure exhibit reduce non-specific binding of proteins, nucleic acids, fluorophores, and other components of biological and biochemical assay methods. The degree of non-specific binding exhibited by a given support surface, e.g., an interior flow cell device surface, may be assessed either qualitatively or quantitatively. For example, in some instances, exposure of the surface to fluorescent dyes (e.g., cyanine dye 3 (Cy3), cyanine dye 5 (Cy5), etc.), fluorescently-labeled nucleotides, fluorescently-labeled oligonucleotides, and/or fluorescently-labeled proteins (e.g. polymerases) under a standardized set of conditions, followed by a specified rinse protocol and fluorescence imaging may be used as a qualitative tool for comparison of non-specific binding on supports comprising different surface formulations. In some instances, exposure of the surface to fluorescent dyes, fluorescently-labeled nucleotides, fluorescently-labeled oligonucleotides, and/or fluorescently-labeled proteins (e.g. polymerases) under a standardized set of conditions, followed by a specified rinse protocol and fluorescence imaging may be used as a quantitative tool for comparison of non-specific binding on supports comprising different surface formulations—provided that care has been taken to ensure that the fluorescence imaging is performed under conditions where fluorescence signal is linearly related (or related in a predictable manner) to the number of fluorophores on the support surface (e.g., under conditions where signal saturation and/or self-quenching of the fluorophore is not an issue) and suitable calibration standards are used. In some instances, other techniques known to those of skill in the art, for example, radioisotope labeling and counting methods may be used for quantitative assessment of the degree to which non-specific binding is exhibited by the different support surface formulations of the present disclosure.

In some instances, the degree of non-specific binding exhibited by the disclosed low nonspecific binding support surfaces may be assessed using a standardized protocol for contacting the surface with a labeled protein (e.g., bovine serum albumin (BSA), streptavidin, a DNA polymerase, a reverse transcriptase, a helicase, a single-stranded binding protein (SSB), etc., or any combination thereof), a labeled nucleotide, a labeled oligonucleotide, etc., under a standardized set of incubation and rinse conditions, followed by detection of the amount of label remaining on the surface and comparison of the signal resulting therefrom to an appropriate calibration standard. In some instances, the label may comprise a fluorescent label. In some instances, the label may comprise a radioisotope. In some instances, the label may comprise any other detectable label known to one of skill in the art. In some instances, the degree of non-specific binding exhibited by a given support surface formulation may thus be assessed in terms of the number of non-specifically bound protein molecules (or other molecules) per unit area. In some instances, the low nonspecific binding supports of the present disclosure may exhibit nonspecific protein binding (or nonspecific binding of other specified molecules, e.g., cyanine dye 3 (Cy3) of less than 0.001 molecule per μm^2 , less than 0.01 molecule per μm^2 ,

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less than 0.1 molecule per μm^2 , less than 0.25 molecule per μm^2 , less than 0.5 molecule per μm^2 , less than 1 molecule per μm^2 , less than 10 molecules per μm^2 , less than 100 molecules per μm^2 , or less than 1,000 molecules per μm^2 .

Those of skill in the art will realize that a given support surface of the present disclosure may exhibit nonspecific binding falling anywhere within this range, for example, of less than 86 molecules per μm^2 . For example, some modified surfaces disclosed herein exhibit nonspecific protein binding of less than 0.5 molecule/ μm^2 following contact with a 1 uM solution of Cy3 labeled streptavidin (GE Amersham) in phosphate buffered saline (PBS) buffer for 15 minutes, followed by 3 rinses with deionized water. Some modified surfaces disclosed herein exhibit nonspecific binding of Cy3 dye molecules of less than 0.25 molecules per μm^2 . In independent nonspecific binding assays, 1 μM labeled Cy3 SA (ThermoFisher), 1 uM Cy5 SA dye (ThermoFisher), 10 uM Aminoallyl-dUTP-ATTO-647N (Jena Biosciences), 10 uM Aminoallyl-dUTP-ATTO-Rho11 (Jena Biosciences), 10 uM Aminoallyl-dUTP-ATTO-Rho11 (Jena Biosciences), 10 uM 7-Propargylamino-7-deaza-dGTP-Cy5 (Jena Biosciences), and 10 uM 7-Propargylamino-7-deaza-dGTP-Cy3 (Jena Biosciences) were incubated on the low nonspecific binding substrates at 37° C. for 15 minutes in a 384 well plate format. Each well was rinsed 2-3× with 50 ul deionized RNase/DNase Free water and 2-3× with 25 mM ACES buffer pH 7.4. The 384 well plates were imaged on a GE Typhoon (GE Healthcare Lifesciences, Pittsburgh, Pa.) instrument using the Cy3, AF555, or Cy5 filter sets (according to dye test performed) as specified by the manufacturer at a PMT gain setting of 800 and resolution of 50-100 μm . For higher resolution imaging, images were collected on an Olympus IX83 microscope (Olympus Corp., Center Valley, Pa.) with a total internal reflectance fluorescence (TIRF) objective (20×, 0.75 NA or 100×, 1.5 NA, Olympus), an sCMOS Andor camera (Zyla 4.2. Dichroic mirrors were purchased from Semrock (IDEX Health & Science, LLC, Rochester, N.Y.), e.g., 405, 488, 532, or 633 nm dichroic reflectors/beamsplitters, and band pass filters were chosen as 532 LP or 645 LP concordant with the appropriate excitation wavelength. Some modified surfaces disclosed herein exhibit nonspecific binding of dye molecules of less than 0.25 molecules per μm^2 .

In some instances, the coated flow cell device surfaces disclosed herein may exhibit a ratio of specific to nonspecific binding of a fluorophore such as Cy3 of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 50, 75, 100, or greater than 100, or any intermediate value spanned by the range herein.

In some instances, one or more surface modification and/or polymer layers may be applied to the interior flow cell device surfaces using a technique such as chemical vapor deposition (CVD). In some instances, one or more surface modification and/or polymer layers may be applied to the interior flow cell device surfaces by flowing one or more appropriate chemical coupling or coating reagents through the capillaries or fluid channels prior to use for their intended application. In some instances, one or more coating reagents may be added to a buffer used, e.g., a nucleic acid hybridization, amplification reaction, and/or sequencing reaction buffer to provide for dynamic coating of the interior flow cell device surfaces.

In some instances, the chemical modification layers may be applied uniformly across the surface of the substrate or support structure. Alternately, the surface of the substrate or support structure may be non-uniformly distributed or patterned, such that the chemical modification layers are con-

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fined to one or more discrete regions of the substrate. For example, the substrate surface may be patterned using photolithographic techniques to create an ordered array or random pattern of chemically-modified regions on the surface. Alternately or in combination, the substrate surface may be patterned using, e.g., contact printing and/or ink-jet printing techniques. In some instances, an ordered array or random patter of chemically-modified discrete regions may comprise at least 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, or 10,000 or more discrete regions, or any intermediate number of discrete regions spanned by the range herein.

In some instances, fluorescence images of the disclosed low nonspecific binding surfaces when used, e.g., in nucleic acid hybridization or amplification applications to create clusters of hybridized or clonally-amplified nucleic acid molecules (e.g., “discrete regions” that have been directly or indirectly labeled with a fluorophore) exhibit contrast-to-noise ratios (CNRs) of at least 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 20, 210, 220, 230, 240, 250, or greater than 250 when the nucleic acid molecules are labeled with Cy3 and the images are acquired using an Olympus IX83 inverted fluorescence microscope equipped with a 20×, 0.75 NA objective, a 532 nm light source, a bandpass and dichroic mirror filter set adapted or optimized for 532 nm long-pass excitation and Cy3 fluorescence emission filter, a Semrock 532 nm dichroic reflector, and a camera (Andor sCMOS, Zyla 4.2) where the excitation light intensity is adjusted to avoid signal saturation, and the surface is immersed in a buffer (e.g., 25 mM ACES, pH 7.4 buffer) while the image is acquired. As used herein, contrast-to-noise ratio (CNR) is calculated as:

$$\text{CNR}=(S-B)/\text{Noise}$$

where S=foreground signal (e.g., the fluorescence signal as measured in the image that arises from a labeled nucleic acid colony or cluster on a sample support surface), B=background signal (where $B=B_{inter}+B_{intra}$), B_{inter} =background signal measured at a location on the sample support surface that is between labeled nucleic acid colonies or clusters, B_{intra} =background signal measured at the location of a nucleic acid colony or cluster (determined, e.g., by contacting the sample support surface with a labeled, non-complementary oligonucleotide and measuring the resulting fluorescence), and Noise=the signal noise. The contrast-to-noise ratio (CNR) of images of sequencing surfaces, for example, provides a key metric in assessing nucleic acid amplification specificity and non-specific binding on the support. While signal-to-noise ratio (SNR) is often considered to be a benchmark of overall signal quality, it can be shown that improved CNR can provide a significant advantage over SNR as a benchmark for signal quality in imaging applications that require rapid image capture (e.g., nucleic acid sequencing applications for which cycle times must be minimized).

In some instances, polymer-coated sample support structures, e.g., interior flow cell device surfaces comprising the disclosed hydrophilic polymer coatings, may exhibit improved stability to repetitive exposure to solvents, changes in temperature, changes in pH, or long-term storage.

Fluidics Systems and Fluid Flow Control Modules:

in some implementations, the disclosed imaging and/or analysis systems may provide fluid flow control capability for delivering samples or reagents to the one or more flow cell devices or flow cell cartridges (e.g., single capillary flow

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cell device or microfluidic channel flow cell device) connected to the system. Reagents and buffers may be stored in bottles, reagent and buffer cartridges, or other suitable containers that are connected to the flow cell inlets by means of tubing and valve manifolds. The disclosed systems may also include processed sample and waste reservoirs in the form of bottles, cartridges, or other suitable containers for collecting fluids downstream of the capillary flow cell devices or capillary flow cell cartridges. In some embodiments, the fluid flow (or “fluidics”) control module may provide programmable switching of flow between different sources, e.g. sample or reagent reservoirs or bottles located in the instrument, and the inlet(s) to a central region (e.g., a capillary flow cell or microfluidic device, or a large fluid chamber such as a large fluid chamber within a microfluidic device). In some instances, the fluid flow control module may provide programmable switching of flow between outlet(s) from the central region (e.g., a capillary flow cell or microfluidic device) and different collection points, e.g., processed sample reservoirs, waste reservoirs, etc., connected to the system. In some instances, samples, reagents, and/or buffers may be stored within reservoirs that are integrated into the flow cell cartridge or microfluidic cartridge itself. In some instances, processed samples, spent reagents, and/or used buffers may be stored within reservoirs that are integrated into the flow cell cartridge or microfluidic device cartridge itself.

In some implementations, one or more fluid flow control modules may be configured to control the delivery of fluids to one or more capillary flow cells, capillary flow cell cartridges, microfluidic devices, microfluidic cartridges, or any combination thereof. In some instances, the one or more fluidics controllers may be configured to control volumetric flow rates for one or more fluids or reagents, linear flow velocities for one or more fluids or reagents, mixing ratios for one or more fluids or reagents, or any combination thereof. Control of fluid flow through the disclosed systems will typically be performed using pumps (or other fluid actuation mechanisms) and valves (e.g., programmable pumps and valves). Examples of suitable pumps include, but are not limited to, syringe pumps, programmable syringe pumps, peristaltic pumps, diaphragm pumps, and the like. Examples of suitable valves include, but are not limited to, check valves, electromechanical two-way or three-way valves, pneumatic two-way and three-way valves, and the like. In some instances, fluid flow through the system may be controlled by means of applying positive pneumatic pressure to one or more inlets of the reagent and buffer containers, or to inlets incorporated into flow cell cartridge(s) (e.g., capillary flow cell or microfluidic cartridges). In some embodiments, fluid flow through the system may be controlled by means of drawing a vacuum at one or more outlets of waste reservoir(s), or at one or more outlets incorporated into flow cell cartridge(s) (e.g., capillary flow cell or microfluidic cartridges).

In some instances, different modes of fluid flow control are utilized at different points in an assay or analysis procedure, e.g. forward flow (relative to the inlet and outlet for a given capillary flow cell device), reverse flow, oscillating or pulsatile flow, or combinations thereof. In some applications, oscillating or pulsatile flow may be applied, for example, during assay wash/rinse steps to facilitate complete and efficient exchange of fluids within the one or more flow cell devices or flow cell cartridges (e.g., capillary flow cell devices or cartridges, and microfluidic devices or cartridges).

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Similarly, in some cases different fluid flow rates may be utilized at different locations within a flow cell device or at different points in the assay or analysis process workflow, for example, in some instances, the volumetric flow rate may vary from -100 ml/sec to +100 ml/sec. In some embodiment, the absolute value of the volumetric flow rate may be at least 0.001 ml/sec, at least 0.01 ml/sec, at least 0.1 ml/sec, at least 1 ml/sec, at least 10 ml/sec, or at least 100 ml/sec. In some embodiments, the absolute value of the volumetric flow rate may be at most 100 ml/sec, at most 10 ml/sec, at most 1 ml/sec, at most 0.1 ml/sec, at most 0.01 ml/sec, or at most 0.001 ml/sec. The volumetric flow rate at a given location with the flow cell device or at a given point in time may have any value within this range, e.g. a forward flow rate of 2.5 ml/sec, a reverse flow rate of -0.05 ml/sec, or a value of 0 ml/sec (i.e., stopped flow).

In some implementations, the fluidics system may be designed to minimize the consumption of key reagents (e.g., expensive reagents) required for performing, e.g., genomic analysis applications. For example, in some implementations the disclosed fluidics systems may comprise a first reservoir housing a first reagent or solution, a second reservoir housing a second reagent or solution, and a central region, e.g., a central capillary flow cell or microfluidic device, where an outlet from the first reservoir and an outlet from the second reservoir are fluidically coupled to an inlet of the central capillary flow cell or microfluidic device through at least one valve such that the volume of the first reagent or solution flowing per unit time from the outlet of the first reservoir to the inlet of the central capillary flow cell or microfluidic device is less than the volume of the second reagent or solution flowing per unit time from the outlet of the second reservoir to the inlet of the central region. In some implementations, the first reservoir and second reservoir may be integrated into a capillary flow cell cartridge or microfluidic cartridge. In some instances, the at least one valve may also be integrated into the capillary flow cell cartridge or microfluidic cartridge.

In some instances, the first reservoir is fluidically coupled to the central capillary flow cell or microfluidic device through a first valve, and the second reservoir is fluidically coupled to the central capillary flow cell or microfluidic device through a second valve. In some instances, the first and/or second valves may be, e.g., a diaphragm valve, pinch valve, gate valve, or other suitable valve. In some instances, the first reservoir is positioned in close proximity to the inlet of the central capillary flow cell or microfluidic device to reduce dead volume for delivery of the first reagent solution. In some instances, the first reservoir is placed in closer proximity to the inlet of the central capillary flow cell or microfluidic device than is the second reservoir. In some instances, the first reservoir is positioned in close proximity to the second valve so as to reduce the dead volume for delivery of the first reagent relative to that for delivery of a plurality of "second" reagents (e.g., two, three, four, five, or six or more "second" reagents) from a plurality of "second" reservoirs (e.g., two, three, four, five, or six or more "second" reservoirs).

The first and second reservoirs described above may be used to house the same or different reagents or solutions. In some instances, the first reagent that is housed in the first reservoir is different from the second reagent that is housed in the second reservoir, and the second reagent comprises at least one reagent that is used in common by a plurality of reactions occurring in the central a central capillary flow cell or microfluidic device. In some instances, e.g., in fluidics systems configured for performing nucleic acid sequencing

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chemistry within the central capillary flow cell or microfluidic device, the first reagent comprises at least one reagent selected from the group consisting of a polymerase, nucleotide, and a nucleotide analog. In some instances, the second reagent comprises a low-cost reagent, e.g., a solvent.

In some instances, the interior volume of the central region, e.g., a central capillary flow cell cartridge, or microfluidic device comprising one or more fluid channels or fluid chambers, can be adjusted based on the specific application to be performed, e.g., nucleic acid sequencing. In some embodiments, the central region comprises an interior volume suitable for sequencing a eukaryotic genome. In some embodiments, the central region comprises an interior volume suitable for sequencing a prokaryotic genome. In some embodiments, the central region comprises an interior volume suitable for sequencing a viral genome. In some embodiments, the central region comprises an interior volume suitable for sequencing a transcriptome. For example, in some embodiments, the interior volume of the central region may comprise a volume of less than 0.05 μ l, between 0.05 μ l and 0.1 μ l, between 0.05 μ l and 0.2 μ l, between 0.05 μ l and 0.5 μ l, between 0.05 μ l and 0.8 μ l, between 0.05 μ l and 1 μ l, between 0.05 μ l and 1.2 μ l, between 0.05 μ l and 1.5 μ l, between 0.1 μ l and 1.5 μ l, between 0.2 μ l and 1.5 μ l, between 0.5 μ l and 1.5 μ l, between 0.8 μ l and 1.5 μ l, between 1 μ l and 1.5 μ l, between 1.2 μ l and 1.5 μ l, or greater than 1.5 μ l, or a range defined by any two of the foregoing. In some embodiments, the interior volume of the central region may comprise a volume of less than 0.5 μ l, between 0.5 μ l and 1 μ l, between 0.5 μ l and 2 μ l, between 0.5 μ l and 5 μ l, between 0.5 μ l and 8 μ l, between 0.5 μ l and 10 μ l, between 0.5 μ l and 12 μ l, between 0.5 μ l and between 1 μ l and 15 μ l, between 2 μ l and 15 μ l, between 5 μ l and 15 μ l, between 8 μ l and 15 μ l, between 10 μ l and 15 μ l, between 12 μ l and 15 μ l, or greater than 15 μ l, or a range defined by any two of the foregoing. In some embodiments, the interior volume of the central region may comprise a volume of less than 5 μ l, between 5 μ l and 10 μ l, between 5 μ l and 20 μ l, between 5 μ l and 500 μ l, between 5 μ l and 80 μ l, between 5 μ l and 100 μ l, between 5 μ l and 120 μ l, between 5 μ l and 150 μ l, between 10 μ l and 150 μ l, between 20 μ l and 150 μ l, between 50 μ l and 150 μ l, between 80 μ l and 150 μ l, between 100 μ l and 150 μ l, between 120 μ l and 150 μ l, or greater than 150 μ l, or a range defined by any two of the foregoing. In some embodiments, the interior volume of the central region may comprise a volume of less than 50 μ l, between 50 μ l and 100 μ l, between 50 μ l and 200 μ l, between 50 μ l and 500 μ l, between 50 μ l and 800 μ l, between 50 μ l and 1000 μ l, between 50 μ l and 1200 μ l, between 50 μ l and 1500 μ l, between 100 μ l and 1500 μ l, between 200 μ l and 1500 μ l, between 500 μ l and 1500 μ l, between 800 μ l and 1500 μ l, between 1000 μ l and 1500 μ l, between 1200 μ l and 1500 μ l, or greater than 1500 μ l, or a range defined by any two of the foregoing. In some embodiments, the interior volume of the central region may comprise a volume of less than 500 μ l, between 500 μ l and 1000 μ l, between 500 μ l and 2000 μ l, between 500 μ l and 5 ml, between 500 μ l and 8 ml, between 500 μ l and 10 ml, between 500 μ l and 12 ml, between 500 μ l and 15 ml, between 1 ml and 15 ml, between 2 ml and 15 ml, between 5 ml and 15 ml, between 8 ml and 15 ml, between 10 ml and 15 ml, between 12 ml and 15 ml, or greater than 15 ml, or a range defined by any two of the foregoing. In some embodiments, the interior volume of the central region may comprise a volume of less than 5 ml, between 5 ml and 10 ml, between 5 ml and 20 ml, between 5 ml and 50 ml, between 5 ml and 80 ml, between 5 ml and 100 ml, between 5 ml and 120 ml, between 5 ml and 150 ml, between 10 ml and 150 ml, between 20 ml and 150 ml, between 50 ml and

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150 ml, between 80 ml and 150 ml, between 100 ml and 150 ml, between 120 ml and 150 ml, or greater than 150 ml, or a range defined by any two of the foregoing. In some embodiments, the systems described herein comprise an array or collection of flow cell devices or systems comprising multiple discrete capillaries, microfluidic channels, fluidic channels, chambers, or luminal regions, wherein the combined interior volume is, comprises, or includes one or more of the values within a range disclosed herein.

In some instances, the ratio of volumetric flow rate for the delivery of the first reagent to the central capillary flow cell or microfluidic device to that for delivery of the second reagent to the central capillary flow cell or microfluidic device may be less than 1:20, less than 1:16, least than 1:12, less than 1:10, less than 1:8, less than 1:6, or less than 1:2. In some instances, the ratio of volumetric flow rate for the delivery of the first reagent to the central capillary flow cell or microfluidic device to that for delivery of the second reagent to the central capillary flow cell or microfluidic device may have any value with the range spanned by these values, e.g., less than 1:15.

As noted, the flow cell devices and/or fluidics systems disclosed herein may be configured to achieve a more efficient use of the reagents than that achieved by, e.g., other sequencing devices and systems, particularly for the costly reagents used in a variety of sequencing chemistry steps. In some instances, the first reagent comprises a reagent that is more expensive than the second reagent. In some instances, the first reagent comprises a reaction-specific reagent and the second reagent comprises a nonspecific reagent common to all reactions performed in the central capillary flow cell or microfluidic device region, and wherein the reaction specific reagent is more expensive than the nonspecific reagent.

In some instances, utilization of the flow cell devices and/or fluidic systems disclosed herein may convey advantages in terms of reduced consumption of costly reagents. In some instances, for example, utilization of the flow cell devices and/or fluidic systems disclosed herein may result in at least a 5%, at least a 7.5%, at least a 10%, at least a 12.5%, at least a 15%, at least a 17.5%, at least a 20%, at least a 22.5%, at least a 25%, at least a 30%, at least a 35%, at least a 40%, at least a 45%, or at least a 50% reduction in reagent consumption compared to the reagent consumption encountered when operating, e.g., current commercially-available nucleic acid sequencing systems.

FIG. 31 illustrates a non-limiting example of a simple fluidics system comprising a single capillary flow cell connected to various fluid flow control components, where the single capillary is optically accessible and compatible with mounting on a microscope stage or in a custom imaging instrument for use in various imaging applications. A plurality of reagent reservoirs is fluidically-coupled with the inlet end of the single capillary flow cell device, where the reagent flowing through the capillary at any given point in time is controlled by means of a programmable rotary valve that allows the user to control the timing and duration of reagent flow. In this non-limiting example, fluid flow is controlled by means of a programmable syringe pump that provides precise control and timing of volumetric fluid flow and fluid flow velocity.

FIG. 32 illustrates a non-limiting example of a fluidics system that comprises a capillary flow cell cartridge having integrated diaphragm valves to reduce or minimize dead volume and conserve certain key reagents. The integration of miniature diaphragm valves into the cartridge allows the valve to be positioned in close proximity to the inlet of the capillary, thereby reducing or minimizing dead volume

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within the device and reducing the consumption of costly reagents. The integration of valves and other fluid control components within the capillary flow cell cartridge also allows greater fluid flow control functionality to be incorporated into the cartridge design.

FIG. 33 illustrates a non-limiting example of a capillary flow cell cartridge-based fluidics system used in combination with a microscope setup, where the cartridge incorporates or mates with a temperature control component such as a metal plate that makes contact with the capillaries within the cartridge and serves as a heat source/sink. The microscope setup consists of an illumination system (e.g., including a laser, LED, or halogen lamp, etc., as a light source), an objective lens, an imaging system (e.g., a CMOS or CCD camera), and a translation stage to move the cartridge relative to the optical system, which allows, e.g., fluorescence and/or bright field images to be acquired for different regions of the capillary flow cells as the stage is moved.

Temperature Control Modules:

In some implementations the disclosed systems will include temperature control functionality for the purpose of facilitating the accuracy and reproducibility of assay or analysis results. Examples of temperature control components that may be incorporated into the instrument system (or capillary flow cell cartridge) design include, but are not limited to, resistive heating elements, infrared light sources, Peltier heating or cooling devices, heat sinks, thermistors, thermocouples, and the like. In some instances, the temperature control module (or "temperature controller") may provide for a programmable temperature change at a specified, adjustable time prior to performing specific assay or analysis steps. In some instances, the temperature controller may provide for programmable changes in temperature over specified time intervals. In some embodiments, the temperature controller may further provide for cycling of temperatures between two or more set temperatures with specified frequency and ramp rates so that thermal cycling for amplification reactions may be performed.

FIG. 34 illustrates one non-limiting example for temperature control of the flow cells (e.g., capillary flow cells or microfluidic device-based flow cells) through the use of a metal plate that is placed in contact with the flow cell cartridge. In some instances, the metal plate may be integrated with the cartridge chassis. In some instances, the metal plate may be temperature controlled using a Peltier or resistive heater.

FIG. 35 illustrates one non-limiting approach for temperature control of the flow cells (e.g., capillary or microfluidic channel flow cells) that comprises a non-contact thermal control mechanism. In this approach, a stream of temperature-controlled air is directed through the flow cell cartridge (e.g., towards a single capillary flow cell device or a microfluidic channel flow cell device) using an air temperature control system. The air temperature control system comprises a heat exchanger, e.g., a resistive heater coil, fins attached to a Peltier device, etc., that is capable of heating and/or cooling the air and holding it at a constant, user-specified temperature. The air temperature control system also comprises an air delivery device, such as a fan, that directs the stream of heated or cooled air to the capillary flow cell cartridge. In some instances, the air temperature control system may be set to a constant temperature T1 so that the air stream, and consequently the flow cell or cartridge (e.g., capillary flow cell or microfluidic channel flow cell) is kept at a constant temperature T2, which in some cases may differ from the set temperature T1 depending on the environment temperature, air flow rate, etc. In

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some instances, two or more such air temperature control systems may be installed around the capillary flow cell device or flow cell cartridge so that the capillary or cartridge may be rapidly cycled between several different temperatures by controlling which one of the air temperature control systems is active at a given time. In another approach, the temperature setting of the air temperature control system may be varied so the temperature of the capillary flow cell or cartridge may be changed accordingly.

Fluid Dispensing Robotics:

In some implementations, the disclosed systems may comprise an automated, programmable fluid-dispensing (or liquid-dispensing) system for use in dispensing reagents or other solutions into, e.g., microplates, capillary flow cell devices and cartridges, microfluidic devices and cartridges, etc. Suitable automated, programmable fluid-dispensing systems are commercially available from a number of vendors, e.g. Beckman Coulter, Perkin Elmer, Tecan, Velocity 11, and many others. In a preferred aspect of the disclosed systems, the fluid-dispensing system further comprises a multichannel dispense head, e.g. a 4 channel, 8 channel, 16 channel, 96 channel, or 384 channel dispense head, for simultaneous delivery of programmable volumes of liquid (e.g. ranging from about 1 microliter to several milliliters) to multiple wells or locations on a flow cell cartridge or microfluidic cartridge.

Cartridge- and/or Microplate-Handling (Pick-and-Place) Robotics:

In some implementations, the disclosed system may comprise a cartridge- and/or microplate-handling robotic system for automated replacement and positioning of microplates, capillary flow cell cartridges, or microfluidic device cartridges in relation to the optical imaging system, or for optionally moving microplates, capillary flow cell cartridges, or microfluidic device cartridges between the optical imaging system and a fluid-dispensing system. Suitable automated, programmable microplate-handling robotic systems are commercially available from a number of vendors, including Beckman Coulter, Perkin Elmer, Tecan, Velocity 11, and many others. In a preferred aspect of the disclosed systems, an automated microplate-handling robotic system is configured to move collections of microwell plates comprising samples and/or reagents to and from, e.g., refrigerated storage units.

Spectroscopy or Imaging Modules:

As indicated above, in some implementations the disclosed analysis systems will include optical imaging capabilities and may also include other spectroscopic measurement capabilities. For example, the disclosed imaging modules may be configured to operate in any of a variety of imaging modes known to those of skill in the art including, but not limited to, bright-field, dark-field, fluorescence, luminescence, or phosphorescence imaging. In some instances, the one or more capillary flow cells or microfluidic devices of a fluidics sub-system comprise a window that allows at least a section of one or more capillaries or one or more fluid channels in each flow cell or microfluidic device to be illuminated and imaged.

In some embodiments, single wavelength excitation and emission fluorescence imaging may be performed. In some embodiments, dual wavelength excitation and emission (or multi-wavelength excitation or emission) fluorescence imaging may be performed. In some instances, the imaging module is configured to acquire video images. The choice of imaging mode may impact the design of the flow cells devices or cartridges in that all or a portion of the capillaries or cartridge will necessarily need to be optically transparent

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over the spectral range of interest. In some instances, a plurality of capillaries within a capillary flow cell cartridge may be imaged in their entirety within a single image. In some instances, only a single capillary or a subset of capillaries within a capillary flow cell cartridge, or portions thereof, may be imaged within a single image. In some instances, a series of images may be “tiled” to create a single high-resolution image of one, two, several, or the entire plurality of capillaries within a cartridge. In some instances, a plurality of fluid channels within a microfluidic chip may be imaged in their entirety within a single image. In some instances, only a single fluid channel or a subset of fluid channels within a microfluidic chip, or portions thereof, may be imaged within a single image. In some instances, a series of images may be “tiled” to create a single high-resolution image of one, two, several, or the entire plurality of fluid channels within a cartridge.

A spectroscopy or imaging module may comprise, e.g., a microscope equipped with a CMOS or CCD camera. In some instances, the spectroscopy or imaging module may comprise, e.g., a custom instrument such as one of the imaging modules described herein that is configured to perform a specific spectroscopic or imaging technique of interest. In general, the hardware associated with the spectroscopy or imaging module may include light sources, detectors, and other optical components, as well as processors or computers.

Light Sources:

Any of a variety of light sources may be used to provide the imaging or excitation light, including but not limited to, tungsten lamps, tungsten-halogen lamps, arc lamps, lasers, light emitting diodes (LEDs), or laser diodes. In some instances, a combination of one or more light sources, and additional optical components, e.g. lenses, filters, apertures, diaphragms, mirrors, and the like, may be configured as an illumination system (or sub-system).

Detectors:

Any of a variety of image sensors may be used for imaging purposes, including but not limited to, photodiode arrays, charge-coupled device (CCD) cameras, or complementary metal-oxide-semiconductor (CMOS) image sensors. As used herein, “imaging sensors” may be one-dimensional (linear) or two-dimensional array sensors. In many instances, a combination of one or more image sensors, and additional optical components, e.g. lenses, filters, apertures, diaphragms, mirrors, and the like, may be configured as an imaging system (or sub-system). In some instances, e.g., where spectroscopic measurements are performed by the system rather than imaging, suitable detectors may include, but are not limited to, photodiodes, avalanche photodiodes, and photomultipliers.

Other Optical Components:

The hardware components of the spectroscopic measurement or imaging module may also include a variety of optical components for steering, shaping, filtering, or focusing light beams through the system. Examples of suitable optical components include, but are not limited to, lenses, mirrors, prisms, apertures, diffraction gratings, colored glass filters, long-pass filters, short-pass filters, bandpass filters, narrowband interference filters, broadband interference filters, dichroic reflectors, optical fibers, optical waveguides, and the like. In some instances, as noted above, the spectroscopic measurement or imaging module may further comprise one or more translation stages or other motion control mechanisms for the purpose of moving capillary flow cell devices and cartridges relative to the illumination and/or detection/imaging sub-systems, or vice versa.

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Total Internal Reflection:

In some instances, the optical module or sub-system may be designed to use all or a portion of an optically transparent wall of the capillaries or microfluidic channels in flow cell devices and cartridges as a waveguide for delivering excitation light to the capillary or channel lumen(s) via total internal reflection. When incident excitation light strikes the surface of the capillary or channel lumen at an angle with respect to a normal to the surface that is larger than the critical angle (determined by the relative refractive indices of the capillary or channel wall material and the aqueous buffer within the capillary or channel), total internal reflection occurs at the surface and the light propagates through the capillary or channel wall along the length of the capillary or channel. Total internal reflection generates an evanescent wave at the lumen surface which penetrates the lumen interior for extremely short distances, and which may be used to selectively excite fluorophores at the surface, e.g., labeled nucleotides that have been incorporated by a polymerase into a growing oligonucleotide through a solid-phase primer extension reaction.

Light-Tight Housings and Environmental Control Chambers:

In some implementations, the disclosed systems may comprise a light-tight housing to prevent stray ambient light from creating glare and obscuring, e.g., relatively faint fluorescence signals. In some implementations, the disclosed systems may comprise an environmental control chamber that enables the system to operate under a tightly controlled temperature, humidity level, etc.

Processors and Computers:

In some instances, the disclosed systems may comprise one or more processors or computers. The processor may be a hardware processor such as a central processing unit (CPU), a graphic processing unit (GPU), a general-purpose processing unit, or a computing platform. The processor may be comprised of any of a variety of suitable integrated circuits, microprocessors, logic devices, field-programmable gate arrays (FPGAs) and the like. In some instances, the processor may be a single core or multi core processor, or a plurality of processors may be configured for parallel processing. Although the disclosure is described with reference to a processor, other types of integrated circuits and logic devices are also applicable. The processor may have any suitable data operation capability. For example, the processor may perform 512 bit, 256 bit, 128 bit, 64 bit, 32 bit, or 16 bit data operations.

The processor or CPU can execute a sequence of machine-readable instructions, which can be embodied in a program or software. The instructions may be stored in a memory location. The instructions can be directed to the CPU, which can subsequently program or otherwise configure the CPU to implement, e.g., the system control methods of the present disclosure. Examples of operations performed by the CPU can include fetch, decode, execute, and write back.

Some processors may comprise a processing unit of a computer system. The computer system may enable cloud-based data storage and/or computing. In some instances, the computer system may be operatively coupled to a computer network ("network") with the aid of a communication interface. The network may be the internet, an intranet and/or extranet, an intranet and/or extranet that is in communication with the internet, or a local area network (LAN). The network in some cases is a telecommunication and/or

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data network. The network may include one or more computer servers, which may enable distributed computing, such as cloud-based computing.

The computer system may also include computer memory or memory locations (e.g., random-access memory, read-only memory, flash memory), electronic storage units (e.g., hard disk), communication interfaces (e.g., network adapters) for communicating with one or more other systems, and peripheral devices, such as cache, other memory units, data storage units and/or electronic display adapters. In some instances, the communication interface may allow the computer to be in communication with one or more additional devices. The computer may be able to receive input data from the coupled devices for analysis. Memory units, storage units, communication interfaces, and peripheral devices may be in communication with the processor or CPU through a communication bus (solid lines), such as may be incorporated into a motherboard. A memory or storage unit may be a data storage unit (or data repository) for storing data. The memory or storage units may store files, such as drivers, libraries and saved programs. The memory or storage units may store user data, e.g., user preferences and user programs.

The system control, image processing, and/or data analysis methods as described herein can be implemented by way of machine-executable code stored in an electronic storage location of the computer system, such as, for example, in the memory or electronic storage unit. The machine-executable or machine-readable code can be provided in the form of software. During use, the code can be executed by the processor. In some cases, the code can be retrieved from the storage unit and stored in memory for ready access by the processor. In some situations, the electronic storage unit can be precluded, and machine-executable instructions are stored in memory.

In some instances, the code may be pre-compiled and configured for use with a machine having a processor adapted to execute the code. In some instances, the code may be compiled during runtime. The code can be supplied in a programming language that can be selected to enable the code to execute in a pre-compiled or as-compiled fashion.

Some aspects of the systems and methods provided herein can be embodied in software. Various aspects of the technology may be thought of as "products" or "articles of manufacture" typically in the form of machine (or processor) executable code and/or associated data that is carried on or embodied in a type of machine-readable medium. Machine-executable code can be stored on an electronic storage unit, such as memory (e.g., read-only memory, random-access memory, flash memory) or a hard disk. "Storage" type media can include any or all of the tangible memory of the computers, processors or the like, or associated modules thereof, such as various semiconductor memories, tape drives, disk drives and the like, which may provide non-transitory storage at any time for the software programming. All or portions of the software may at times be communicated through the Internet or various other telecommunication networks. Such communications, for example, may enable loading of the software from one computer or processor into another, for example, from a management server or host computer into the computer platform of an application server. Thus, another type of media that may bear the software elements includes optical, electrical and electromagnetic waves, such as used across physical interfaces between local devices, through wired and optical landline networks and over various air-links. The physical elements that carry such waves, such as wired or

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wireless links, optical links or the like, also may be considered as media bearing the software. As used herein, unless restricted to non-transitory, tangible “storage” media, terms such as computer or machine “readable medium” refer to any medium that participates in providing instructions to a processor for execution.

In some instances, the system control, image processing, and/or data analysis methods of the present disclosure may be implemented by way of one or more algorithms. An algorithm may be implemented by way of software upon execution by the central processing unit.

System Control Software:

In some instances, the system may comprise a computer (or processor) and a computer-readable medium that includes code for providing a user interface as well as manual, semi-automated, or fully-automated control of all system functions, e.g., control of the fluid flow control module(s), the temperature control module(s), and/or the spectroscopy or imaging module(s), as well as other data analysis and display options. The system computer or processor may be an integrated component of the system (e.g., a microprocessor or mother board embedded within the instrument) or may be a stand-alone module, for example, a main frame computer, a personal computer, or a laptop computer. Examples of fluid flow control functions provided by the system control software include, but are not limited to, volumetric fluid flow rates, fluid flow velocities, the timing and duration for sample and reagent addition, buffer addition, and rinse steps. Examples of temperature control functions provided by the system control software include, but are not limited to, specifying temperature set point(s) and control of the timing, duration, and ramp rates for temperature changes. Examples of spectroscopic measurement or imaging control functions provided by the system control software include, but are not limited to, autofocus capability, control of illumination or excitation light exposure times and intensities, control of image acquisition rate, exposure time, and data storage options.

Image Processing Software:

In some instances, the system may further comprise a computer (or processor) and computer-readable medium that includes code for providing image processing and analysis capability. Examples of image processing and analysis capability that may be provided by the software include, but are not limited to, manual, semi-automated, or fully-automated image exposure adjustment (e.g. white balance, contrast adjustment, signal-averaging and other noise reduction capability, etc.), automated edge detection and object identification (e.g., for identifying clonally-amplified clusters of fluorescently-labeled oligonucleotides on the lumen surface of capillary flow cell devices), automated statistical analysis (e.g., for determining the number of clonally-amplified clusters of oligonucleotides identified per unit area of the capillary lumen surface, or for automated nucleotide base-calling in nucleic acid sequencing applications), and manual measurement capabilities (e.g. for measuring distances between clusters or other objects, etc.). Optionally, instrument control and image processing/analysis software may be written as separate software modules. In some embodiments, instrument control and image processing/analysis software may be incorporated into an integrated package.

Any of a variety of image processing methods known to those of skill in the art may be used for image processing/pre-processing. Examples include, but are not limited to, Canny edge detection methods, Canny-Deriche edge detection methods, first-order gradient edge detection methods (e.g., the Sobel operator), second order differential edge

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detection methods, phase congruency (phase coherence) edge detection methods, other image segmentation algorithms (e.g., intensity thresholding, intensity clustering methods, intensity histogram-based methods, etc.), feature and pattern recognition algorithms (e.g., the generalized Hough transform for detecting arbitrary shapes, the circular Hough transform, etc.), and mathematical analysis algorithms (e.g., Fourier transform, fast Fourier transform, wavelet analysis, auto-correlation, etc.), or any combination thereof.

Nucleic Acid Sequencing Systems & Applications:

Nucleic acid sequencing provides one non-limiting example of an application for the disclosed flow cell devices (e.g., capillary flow cell devices or cartridges and microfluidic devices and cartridges) and imaging systems. Many “second generation” and “third generation” sequencing technologies utilize a massively parallel, cyclic array approach to perform sequencing-by-nucleotide incorporation, in which accurate decoding of a single-stranded template oligonucleotide sequence tethered to a solid support relies on successfully classifying signals that arise from the stepwise addition of A, G, C, and T nucleotides by a polymerase to a complementary oligonucleotide strand. These methods typically require the oligonucleotide template to be modified with a known adapter sequence of fixed length, affixed to a solid support (e.g., the lumen surface(s) of the disclosed capillary flow cell devices or microfluidic chips) in a random or patterned array by hybridization to surface-tethered capture probes (also referred to herein as “adapters” or “primers” tethered to the interior flow cell surfaces) of known sequence that are complementary to that of the adapter sequence, and then probed through a cyclic series of single base addition primer extension reactions that use, e.g., fluorescently-labeled nucleotides to identify the sequence of bases in the template oligonucleotides. These processes thus require the use of miniaturized fluidics systems that offer precise, reproducible control of the timing of reagent introduction to the flow cell in which the sequencing reactions are performed, and small volumes to reduce or minimize the consumption of costly reagents.

Existing commercially-available NGS flow cells are constructed from layers of glass that have been etched, lapped, and/or processed by other methods to meet the tight dimensional tolerances required for imaging, cooling, and/or other requirements. When flow cells are used as consumables, the costly manufacturing processes required for their fabrication result in costs per sequencing run that are too high to make sequencing routinely accessible to scientists and medical professionals in the research and clinical fields.

This disclosure provides an example of a low-cost flow cell architecture that includes low cost glass or polymer capillaries or microfluidic channels, fluidics adapters, and cartridge chassis. Utilizing glass or polymer capillaries that are extruded in their final cross-sectional geometry may eliminate the need for multiple high-precision and costly glass manufacturing processes. Robustly constraining the orientation of the capillaries or microfluidic channels and providing convenient fluidic connections using molded plastic and/or elastomeric components further reduces cost. Laser bonding the components of the polymer cartridge chassis provides a fast and efficient means of sealing the capillary or the microfluidic channels and structurally stabilizing the capillaries or channels and flow cell cartridge without requiring the use of fasteners or adhesives.

The disclosed devices and systems may be configured to perform nucleic acid sequencing using any of a variety of “sequencing-by-nucleotide incorporation”, “sequencing-by-

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nucleotide binding”, “sequencing-by-nucleotide base-pairing”, and “sequencing-by-avidity” sequencing biochemistries. The improvements in flow cell device design disclosed herein, e.g., comprising hydrophilic coated surfaces that maximize foreground signals for, e.g., fluorescently-labeled nucleic acid clusters disposed thereon, while minimizing background signal may give rise to improvements in CNR for images used for base-calling purposes, in combination with improvements in optical imaging system design for fast dual-surface flow cell imaging (comprising simultaneous or near-simultaneous imaging of the interior flow cell surfaces) achieved through improved objective lens and/or tube lens designs that provide for larger depth of field and larger fields-of-view, and reduced reagent consumption (achieved through improved flow cell design) may give rise to dramatic improvements in base-calling accuracy, shortened imaging cycle times, shortened overall sequencing reaction cycle times, and higher throughput nucleic acid sequencing at reduced cost per base.

The systems disclosed herein may be configured to implement any of a variety of different sequencing methodologies using a variety of different sequencing chemistries. For example, FIG. 40 provides a non-limiting example of a flow chart for implementing a sequencing-by-avidity method. A polymer-nucleotide conjugate may be used to form a multivalent binding complex with a plurality of primed target nucleic acid sequences tethered to a support surface, e.g., one or more interior surfaces of a flow cell, such that the multivalent binding complex exhibits a significantly longer persistence time than afforded by the binding interactions between single nucleotides and single primed target nucleic acid sequences. In general, such a sequencing-by-avidity approach will comprise one or more of the following steps: hybridization of target nucleic acid sequences to adapter/primer sequences tethered to the support surface; clonal amplification to create clusters of amplified target sequences on the support surface; contacting the support surface with a polymer-nucleotide conjugate comprising a plurality of nucleotide moieties conjugated to a polymer core, wherein the polymer-nucleotide conjugate may further comprise one or more detectable labels, e.g., fluorophores, to create a stable, multivalent binding complex; washing out of any excess, unbound polymer-nucleotide conjugate; detection of multivalent binding complexes, e.g., by fluorescence imaging of the support surface; identification of a nucleotide in the target nucleic acid sequence (base-calling); destabilization of the multivalent binding complex, e.g., by changing the ionic strength, ionic composition, and/or pH of the buffer; rinsing of the flow cell; and performing a primer extension reaction to add a nucleotide comprising the complementary base for the nucleotide that was identified. The cycle may be repeated to identify additional nucleotide bases in the sequence, followed by processing and assembly of the sequence data. In some instances, data processing may comprise calculation of sequencing performance metrics, such as a Q-score, in real-time as the sequencing run is performed or as part of a post-run data processing step.

In some instances, the disclosed hydrophilic, polymer coated flow cell devices used in combination with the optical imaging systems disclosed herein may confer one or more of the following additional advantages for a nucleic acid sequencing system: (i) decreased fluidic wash times (due to reduced non-specific binding, and thus faster sequencing cycle times), (ii) decreased imaging times (and thus faster turnaround times for assay readout and sequencing cycles), (iii) decreased overall work flow time requirements (due to decreased cycle times), (iv) decreased detection instrumen-

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tation costs (due to the improvements in CNR), (v) improved readout (base-calling) accuracy (due to improvements in CNR), (vi) improved reagent stability and decreased reagent usage requirements (and thus reduced reagents costs), and (vii) fewer run-time failures due to nucleic acid amplification failures.

The methods, devices, and systems disclosed herein for performing nucleic acid sequencing are suitable for a variety of sequencing applications and for sequencing nucleic acid molecules derived from any of a variety of samples and sources. Nucleic acids, in some instances, may be extracted from any of a variety of biological samples, e.g., blood samples, saliva samples, urine samples, cell samples, tissue samples, and the like. For example, the disclosed devices and systems may be used for the analysis of nucleic acid molecules derived from any of a variety of different cell, tissue, or sample types known to those of skill in the art. For example, nucleic acids may be extracted from cells, or tissue samples comprising one or more types of cells, derived from eukaryotes (such as animals, plants, fungi, protista), archaeobacteria, or eubacteria. In some cases, nucleic acids may be extracted from prokaryotic or eukaryotic cells, such as adherent or non-adherent eukaryotic cells. Nucleic acids are variously extracted from, for example, primary or immortalized rodent, porcine, feline, canine, bovine, equine, primate, or human cell lines. Nucleic acids may be extracted from any of a variety of different cell, organ, or tissue types (e.g., white blood cells, red blood cells, platelets, epithelial cells, endothelial cells, neurons, glial cells, astrocytes, fibroblasts, skeletal muscle cells, smooth muscle cells, gametes, or cells from the heart, lungs, brain, liver, kidney, spleen, pancreas, thymus, bladder, stomach, colon, or small intestine). Nucleic acids may be extracted from normal or healthy cells. Alternately or in combination, acids are extracted from diseased cells, such as cancerous cells, or from pathogenic cells that are infecting a host. Some nucleic acids may be extracted from a distinct subset of cell types, e.g., immune cells (such as T cells, cytotoxic (killer) T cells, helper T cells, alpha beta T cells, gamma delta T cells, T cell progenitors, B cells, B-cell progenitors, lymphoid stem cells, myeloid progenitor cells, lymphocytes, granulocytes, Natural Killer cells, plasma cells, memory cells, neutrophils, eosinophils, basophils, mast cells, monocytes, dendritic cells, and/or macrophages, or any combination thereof), undifferentiated human stem cells, human stem cells that have been induced to differentiate, rare cells (e.g., circulating tumor cells (CTCs), circulating epithelial cells, circulating endothelial cells, circulating endometrial cells, bone marrow cells, progenitor cells, foam cells, mesenchymal cells, or trophoblasts). Other cells are contemplated and consistent with the disclosure herein.

Nucleic acids may optionally be attached to one or more non-nucleotide moieties such as labels and other small molecules, large molecules (such as proteins, lipids, sugars, etc.), and solid or semi-solid supports, for example through covalent or non-covalent linkages with either the 5' or 3' end of the nucleic acid. Labels include any moiety that is detectable using any of a variety of detection methods known to those of skill in the art, and thus renders the attached oligonucleotide or nucleic acid similarly detectable. Some labels, e.g., fluorophores, emit electromagnetic radiation that is optically detectable or visible. Alternately or in combination, some labels comprise a mass tag that renders the labeled oligonucleotide or nucleic acid visible in mass spectral data, or a redox tag that renders the labeled oligonucleotide or nucleic acid detectable by amperometry or voltametry. Some labels comprise a magnetic tag that facili-

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tates separation and/or purification of the labeled oligonucleotide or nucleic acid. The nucleotide or polynucleotide is often not attached to a label, and the presence of the oligonucleotide or nucleic acid is directly detected.

Flow Cell Devices Configured for Sequencing:

In some instances, one or more flow cell devices according to the present disclosure may be configured for nucleic acid sequencing applications, e.g., wherein two or more interior flow cell device surfaces comprise hydrophilic polymer coatings that further comprise one or more capture oligonucleotides, e.g., adapter/primer oligonucleotides, or any other oligonucleotides as disclosed herein. In some instances, the hydrophilic, polymer-coated surfaces of the disclosed flow cell devices may comprise a plurality of oligonucleotides tethered thereto that have been selected for use in sequencing a eukaryotic genome. In some instances, the hydrophilic, polymer-coated surfaces of the disclosed flow cell devices may comprise a plurality of oligonucleotides tethered thereto that have been selected for use in sequencing a prokaryotic genome or portion thereof. In some instances, the hydrophilic, polymer-coated surfaces of the disclosed flow cell devices may comprise a plurality of oligonucleotides tethered thereto that have been selected for use in sequencing a viral genome or portion thereof. In some instances, the hydrophilic, polymer-coated surfaces of the disclosed flow cell devices may comprise a plurality of oligonucleotides tethered thereto that have been selected for use in sequencing a transcriptome.

In some instances, a flow cell device of the present disclosure may comprise a first surface in an orientation generally facing the interior of the flow channel, a second surface in an orientation generally facing the interior of the flow channel and further generally facing or parallel to the first surface, a third surface generally facing the interior of a second flow channel, and a fourth surface, generally facing the interior of the second flow channel and generally opposed to or parallel to the third surface; wherein said second and third surfaces may be located on or attached to opposite sides of a generally planar substrate which may be a reflective, transparent, or translucent substrate. In some instances, an imaging surface or imaging surfaces within a flow cell may be located within the center of a flow cell or within or as part of a division between two subunits or subdivisions of a flow cell, wherein said flow cell may comprise a top surface and a bottom surface, one or both of which may be transparent to such detection mode as may be utilized; and wherein a surface comprising oligonucleotides adapters/primers tethered to one or more polymer coatings may be placed or interposed within the lumen of the flow cell. In some instances, the top and/or bottom surfaces do not include attached oligonucleotide adapters/primers. In some instances, said top and/or bottom surfaces do comprise attached oligonucleotide adapters/primers. In some instances, either said top or said bottom surface may comprise attached oligonucleotide adapters/primers. A surface or surfaces placed or interposed within the lumen of a flow cell may be located on or attached to one side, to an opposite side, or to both sides of a generally planar substrate which may be a reflective, transparent, or translucent substrate.

In general, at least one layer of the one or more layers of low nonspecific binding coating on the flow cell device surfaces may comprise functional groups for covalently or non-covalently attaching oligonucleotide molecules, e.g., adapter or primer sequences, or the at least one layer may already comprise covalently or non-covalently attached oligonucleotide adapter or primer sequences at the time that it is deposited on the support surface. In some instances, the

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oligonucleotides tethered to the polymer molecules of at least one third layer may be distributed at a plurality of depths throughout the layer.

In some instances, the oligonucleotide adapter or primer molecules are covalently coupled to the polymer in solution, i.e., prior to coupling or depositing the polymer on the surface. In some instances, the oligonucleotide adapter or primer molecules are covalently coupled to the polymer after it has been coupled to or deposited on the surface. In some instances, at least one hydrophilic polymer layer comprises a plurality of covalently-attached oligonucleotide adapter or primer molecules. In some instances, at least two, at least three, at least four, or at least five layers of hydrophilic polymer comprise a plurality of covalently-attached adapter or primer molecules.

In some instances, the oligonucleotide adapter or primer molecules may be coupled to the one or more layers of hydrophilic polymer using any of a variety of suitable conjugation chemistries known to those of skill in the art. For example, the oligonucleotide adapter or primer sequences may comprise moieties that are reactive with amine groups, carboxyl groups, thiol groups, and the like. Examples of suitable amine-reactive conjugation chemistries that may be used include, but are not limited to, reactions involving isothiocyanate, isocyanate, acyl azide, NHS ester, sulfonyl chloride, aldehyde, glyoxal, epoxide, oxirane, carbonate, aryl halide, imidoester, carbodiimide, anhydride, and fluorophenyl ester groups. Examples of suitable carboxyl-reactive conjugation chemistries include, but are not limited to, reactions involving carbodiimide compounds, e.g., water soluble EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide.HCL). Examples of suitable sulfhydryl-reactive conjugation chemistries include maleimides, haloacetyls and pyridyl disulfides.

One or more types of oligonucleotide molecules may be attached or tethered to the support surface. In some instances, the one or more types of oligonucleotide adapters or primers may comprise spacer sequences, adapter sequences for hybridization to adapter-ligated template library nucleic acid sequences, forward amplification primers, reverse amplification primers, sequencing primers, and/or molecular barcoding sequences, or any combination thereof. In some instances, 1 primer or adapter sequence may be tethered to at least one layer of the surface. In some instances, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 different primer or adapter sequences may be tethered to at least one layer of the surface.

In some instances, the tethered oligonucleotide adapter and/or primer sequences may range in length from about 10 nucleotides to about 100 nucleotides. In some instances, the tethered oligonucleotide adapter and/or primer sequences may be at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, or at least 100 nucleotides in length. In some instances, the tethered oligonucleotide adapter and/or primer sequences may be at most 100, at most 90, at most 80, at most 70, at most 60, at most 50, at most 40, at most 30, at most 20, or at most 10 nucleotides in length. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the length of the tethered oligonucleotide adapter and/or primer sequences may range from about 20 nucleotides to about 80 nucleotides. Those of skill in the art will recognize that the length of the tethered oligonucleotide adapter and/or primer sequences may have any value within this range, e.g., about 24 nucleotides.

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In some instances, the number of coating layers and/or the material composition of each layer is chosen so as to adjust the resultant surface density of oligonucleotide adapters/primers (or other attached molecules) on the coated interior flow cell surfaces. In some instances, the surface density of oligonucleotide adapters/primers may range from about 1,000 primer molecules per μm^2 to about 1,000,000 primer molecules per μm^2 . In some instances, the surface density of oligonucleotide primers may be at least 1,000, at least 10,000, at least 100,000, or at least 1,000,000 molecules per μm^2 . In some instances, the surface density of oligonucleotide primers may be at most 1,000,000, at most 100,000, at most 10,000, or at most 1,000 molecules per μm^2 . Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the surface density of primers may range from about 10,000 molecules per μm^2 to about 100,000 molecules per μm^2 . Those of skill in the art will recognize that the surface density of primer molecules may have any value within this range, e.g., about 455,000 molecules per μm^2 . In some instances, the surface properties of the capillary or channel lumen coating, including the surface density of tethered oligonucleotide primers, may be adjusted to improve or optimize, e.g., solid-phase nucleic acid hybridization specificity and efficiency, and/or solid-phase nucleic acid amplification rate, specificity, and efficiency.

In some instances, the tethered adapter or primer sequences may comprise modifications designed to facilitate the specificity and efficiency of nucleic acid amplification as performed on the low-binding supports. For example, in some instances the primer may comprise polymerase stop points such that the stretch of primer sequence between the surface conjugation point and the modification site is always in single-stranded form and functions as a loading site for 5' to 3' helicases in some helicase-dependent isothermal amplification methods. Other examples of primer modifications that may be used to create polymerase stop points include, but are not limited to, an insertion of a PEG chain into the backbone of the primer between two nucleotides towards the 5' end, insertion of an abasic nucleotide (i.e., a nucleotide that has neither a purine nor a pyrimidine base), or a lesion site which can be bypassed by the helicase.

Nucleic Acid Hybridization:

In some instances, the hydrophilic, polymer coated flow cell device surfaces disclosed herein may provide advantages when used alone or in combination with improved buffer formulations for performing solid-phase nucleic acid hybridization and/or solid-phase nucleic acid amplification reactions as part of genotyping or nucleic acid sequencing applications. In some instances, the polymer-coated flow cell devices disclosed herein may provide advantages in terms of improved nucleic acid hybridization rate and specificity, and improved nucleic acid amplification rates and specificity that may be achieved through one or more of the following additional aspects of the present disclosure: (i) primer design (e.g., sequence and/or modifications), (ii) control of tethered primer density on the solid support, (iii) the surface composition of the solid support, (iv) the surface polymer density of the solid support, (v) the use of improved hybridization conditions before and during amplification, and/or (vi) the use of improved amplification formulations that decrease non-specific primer amplification or increase template amplification efficiency.

In some instances, it may be desirable to vary the surface density of tethered oligonucleotide adapters or primers on the coated flow cell surfaces and/or the spacing of the

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tethered adapters or primers away from the coated flow cell surface (e.g., by varying the length of a linker molecule used to tether the adapter or primers to the surface) in order to “tune” the support for optimal performance when, e.g., using a given amplification method. In some instances, adjusting the surface density of tethered oligonucleotide adapters or primers may impact the level of specific and/or non-specific amplification observed on the surface in a manner that varies according to the amplification method selected. In some instances, the surface density of tethered oligonucleotide adapters or primers may be varied by adjusting the ratio of molecular components used to create the support surface. For example, in the case that an oligonucleotide primer-PEG conjugate is used to create the final layer of a low-binding support, the ratio of the oligonucleotide primer-PEG conjugate to a non-conjugated PEG molecule may be varied. The resulting surface density of tethered primer molecules may then be estimated or measured using any of a variety of techniques known to those of skill in the art. Examples include, but are not limited to, the use of radioisotope labeling and counting methods, covalent coupling of a cleavable molecule that comprises an optically-detectable tag (e.g., a fluorescent tag) that may be cleaved from a support surface of defined area, collected in a fixed volume of an appropriate solvent, and then quantified by comparison of fluorescence signals to that for a calibration solution of known optical tag concentration, or using fluorescence imaging techniques provided that care has been taken with the labeling reaction conditions and image acquisition settings to ensure that the fluorescence signals are linearly related to the number of fluorophores on the surface (e.g., that there is no significant self-quenching of the fluorophores on the surface).

In some instances, the use of the disclosed hydrophilic, polymer-coated flow cell devices, either alone or in combination with improved or optimized buffer formulations, may yield relative hybridization rates that range from about 2× to about 20× faster than that for a conventional hybridization protocol. In some instances, the relative hybridization rate may be at least 2×, at least 3×, at least 4×, at least 5×, at least 6×, at least 7×, at least 8×, at least 9×, at least 10×, at least 12×, at least 14×, at least 16×, at least 18×, at least 20×, at least 25×, at least 30×, or at least 40× that for a conventional hybridization protocol.

In some instances, the use of the disclosed hydrophilic, polymer-coated flow cell devices, either alone or in combination with improved or optimized buffer formulations, may yield total hybridization reaction times (i.e., the time required to reach 90%, 95%, 98%, or 99% completion of the hybridization reaction) of less than 60 minutes, 50 minutes, 40 minutes, 30 minutes, 20 minutes, 15 minutes, 10 minutes, or 5 minutes for any of these completion metrics.

In some instances, the use of the disclosed hydrophilic, polymer-coated flow cell devices, either alone or in combination with improved or optimized buffer formulations, may yield improved hybridization specificity compared to that for a conventional hybridization protocol. In some instances, the hybridization specificity that may be achieved is better than 1 base mismatch in 10 hybridization events, 1 base mismatch in 20 hybridization events, 1 base mismatch in 30 hybridization events, 1 base mismatch in 40 hybridization events, 1 base mismatch in 50 hybridization events, 1 base mismatch in 75 hybridization events, 1 base mismatch in 100 hybridization events, 1 base mismatch in 200 hybridization events, 1 base mismatch in 300 hybridization events, 1 base mismatch in 400 hybridization events, 1 base mismatch in 500 hybridization events, 1 base mismatch in 600

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hybridization events, 1 base mismatch in 700 hybridization events, 1 base mismatch in 800 hybridization events, 1 base mismatch in 900 hybridization events, 1 base mismatch in 1,000 hybridization events, 1 base mismatch in 2,000 hybridization events, 1 base mismatch in 3,000 hybridization events, 1 base mismatch in 4,000 hybridization events, 1 base mismatch in 5,000 hybridization events, 1 base mismatch in 6,000 hybridization events, 1 base mismatch in 7,000 hybridization events, 1 base mismatch in 8,000 hybridization events, 1 base mismatch in 9,000 hybridization events, or 1 base mismatch in 10,000 hybridization events.

In some instances, the use of the disclosed hydrophilic, polymer-coated flow cell devices, either alone or in combination with improved or optimized buffer formulations, may yield improved hybridization efficiency (e.g., the fraction of available oligonucleotide primers on the support surface that are successfully hybridized with target oligonucleotide sequences) compared to that for a conventional hybridization protocol. In some instances, the hybridization efficiency that may be achieved is better than 50%, 60%, 70%, 80%, 85%, 90%, 95%, 98%, or 99% for any of the input target oligonucleotide concentrations specified below and in any of the hybridization reaction times specified above. In some instances, e.g., wherein the hybridization efficiency is less than 100%, the resulting surface density of target nucleic acid sequences hybridized to the support surface may be less than the surface density of oligonucleotide adapter or primer sequences on the surface.

In some instances, use of the disclosed hydrophilic, polymer-coated flow cell devices for nucleic acid hybridization (or nucleic acid amplification) applications using conventional hybridization (or amplification) protocols, or improved or optimized hybridization (or amplification) protocols, may lead to a reduced requirement for the input concentration of target (or sample) nucleic acid molecules contacted with the support surface. For example, in some instances, the target (or sample) nucleic acid molecules may be contacted with the support surface at a concentration ranging from about 10 pM to about 1 μ M (i.e., prior to annealing or amplification). In some instances, the target (or sample) nucleic acid molecules may be administered at a concentration of at least 10 pM, at least 20 pM, at least 30 pM, at least 40 pM, at least 50 pM, at least 100 pM, at least 200 pM, at least 300 pM, at least 400 pM, at least 500 pM, at least 600 pM, at least 700 pM, at least 800 pM, at least 900 pM, at least 1 nM, at least 10 nM, at least 20 nM, at least 30 nM, at least 40 nM, at least 50 nM, at least 60 nM, at least 70 nM, at least 80 nM, at least 90 nM, at least 100 nM, at least 200 nM, at least 300 nM, at least 400 nM, at least 500 nM, at least 600 nM, at least 700 nM, at least 800 nM, at least 900 nM, or at least 1 μ M. In some instances, the target (or sample) nucleic acid molecules may be administered at a concentration of at most 1 μ M, at most 900 nM, at most 800 nm, at most 700 nM, at most 600 nM, at most 500 nM, at most 400 nM, at most 300 nM, at most 200 nM, at most 100 nM, at most 90 nM, at most 80 nM, at most 70 nM, at most 60 nM, at most 50 nM, at most 40 nM, at most 30 nM, at most 20 nM, at most 10 nM, at most 1 nM, at most 900 pM, at most 800 pM, at most 700 pM, at most 600 pM, at most 500 pM, at most 400 pM, at most 300 pM, at most 200 pM, at most 100 pM, at most 90 pM, at most 80 pM, at most 70 pM, at most 60 pM, at most 50 pM, at most 40 pM, at most 30 pM, at most 20 pM, or at most 10 pM. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the target (or

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sample) nucleic acid molecules may be administered at a concentration ranging from about 90 pM to about 200 nM. Those of skill in the art will recognize that the target (or sample) nucleic acid molecules may be administered at a concentration having any value within this range, e.g., about 855 nM.

In some instances, the use of the disclosed hydrophilic, polymer-coated flow cell devices, either alone or in combination with improved or optimized hybridization buffer formulations, may result in a surface density of hybridized target (or sample) oligonucleotide molecules (i.e., prior to performing any subsequent solid-phase or clonal amplification reaction) ranging from about from about 0.0001 target oligonucleotide molecules per μm^2 to about 1,000,000 target oligonucleotide molecules per μm^2 . In some instances, the surface density of hybridized target oligonucleotide molecules may be at least 0.0001, at least 0.0005, at least 0.001, at least 0.005, at least 0.01, at least 0.05, at least 0.1, at least 0.5, at least 1, at least 5, at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 200, at least 300, at least 400, at least 500, at least 600, at least 700, at least 800, at least 900, at least 1,000, at least 1,500, at least 2,000, at least 2,500, at least 3,000, at least 3,500, at least 4,000, at least 4,500, at least 5,000, at least 5,500, at least 6,000, at least 6,500, at least 7,000, at least 7,500, at least 8,000, at least 8,500, at least 9,000, at least 9,500, at least 10,000, at least 15,000, at least 20,000, at least 25,000, at least 30,000, at least 35,000, at least 40,000, at least 45,000, at least 50,000, at least 55,000, at least 60,000, at least 65,000, at least 70,000, at least 75,000, at least 80,000, at least 85,000, at least 90,000, at least 95,000, at least 100,000, at least 150,000, at least 200,000, at least 250,000, at least 300,000, at least 350,000, at least 400,000, at least 450,000, at least 500,000, at least 550,000, at least 600,000, at least 650,000, at least 700,000, at least 750,000, at least 800,000, at least 850,000, at least 900,000, at least 950,000, or at least 1,000,000 molecules per μm^2 . In some instances, the surface density of hybridized target oligonucleotide molecules may be at most 1,000,000, at most 950,000, at most 900,000, at most 850,000, at most 800,000, at most 750,000, at most 700,000, at most 650,000, at most 600,000, at most 550,000, at most 500,000, at most 450,000, at most 400,000, at most 350,000, at most 300,000, at most 250,000, at most 200,000, at most 150,000, at most 100,000, at most 95,000, at most 90,000, at most 85,000, at most 80,000, at most 75,000, at most 70,000, at most 65,000, at most 60,000, at most 55,000, at most 50,000, at most 45,000, at most 40,000, at most 35,000, at most 30,000, at most 25,000, at most 20,000, at most 15,000, at most 10,000, at most 9,500, at most 9,000, at most 8,500, at most 8,000, at most 7,500, at most 7,000, at most 6,500, at most 6,000, at most 5,500, at most 5,000, at most 4,500, at most 4,000, at most 3,500, at most 3,000, at most 2,500, at most 2,000, at most 1,500, at most 1,000, at most 900, at most 800, at most 700, at most 600, at most 500, at most 400, at most 300, at most 200, at most 100, at most 90, at most 80, at most 70, at most 60, at most 50, at most 40, at most 30, at most 20, at most 10, at most 5, at most 1, at most 0.5, at most 0.1, at most 0.05, at most 0.01, at most 0.005, at most 0.001, at most 0.0005, or at most 0.0001 molecules per μm^2 . Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the surface density of hybridized target oligonucleotide molecules may range from about 3,000 molecules per μm^2 to about 20,000 molecules per μm^2 . Those of skill in the art will recognize that the surface

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density of hybridized target oligonucleotide molecules may have any value within this range, e.g., about 2,700 molecules per μm^2 .

Stated differently, in some instances the use of the disclosed low-binding supports alone or in combination with improved or optimized hybridization buffer formulations may result in a surface density of hybridized target (or sample) oligonucleotide molecules (i.e., prior to performing any subsequent solid-phase or clonal amplification reaction) ranging from about 100 hybridized target oligonucleotide molecules per mm^2 to about 1×10^{12} hybridized target oligonucleotide molecules per mm^2 . In some instances, the surface density of hybridized target oligonucleotide molecules may be at least 100, at least 500, at least 1,000, at least 4,000, at least 5,000, at least 6,000, at least 10,000, at least 15,000, at least 20,000, at least 25,000, at least 30,000, at least 35,000, at least 40,000, at least 45,000, at least 50,000, at least 55,000, at least 60,000, at least 65,000, at least 70,000, at least 75,000, at least 80,000, at least 85,000, at least 90,000, at least 95,000, at least 100,000, at least 150,000, at least 200,000, at least 250,000, at least 300,000, at least 350,000, at least 400,000, at least 450,000, at least 500,000, at least 550,000, at least 600,000, at least 650,000, at least 700,000, at least 750,000, at least 800,000, at least 850,000, at least 900,000, at least 950,000, at least 1,000,000, at least 5,000,000, at least 1×10^7 , at least 5×10^7 , at least 1×10^8 , at least 5×10^8 , at least 1×10^9 , at least 5×10^9 , at least 1×10^{10} , at least 5×10^{10} , at least 1×10^{11} , at least 5×10^{11} , or at least 1×10^{12} molecules per mm^2 . In some instances, the surface density of hybridized target oligonucleotide molecules may be at most 1×10^{12} , at most 5×10^{11} , at most 1×10^{11} , at most 5×10^{10} , at most 1×10^{10} , at most 5×10^9 , at most 1×10^9 , at most 5×10^8 , at most 1×10^8 , at most 5×10^7 , at most 1×10^7 , at most 5,000,000, at most 1,000,000, at most 950,000, at most 900,000, at most 850,000, at most 800,000, at most 750,000, at most 700,000, at most 650,000, at most 600,000, at most 550,000, at most 500,000, at most 450,000, at most 400,000, at most 350,000, at most 300,000, at most 250,000, at most 200,000, at most 150,000, at most 100,000, at most 95,000, at most 90,000, at most 85,000, at most 80,000, at most 75,000, at most 70,000, at most 65,000, at most 60,000, at most 55,000, at most 50,000, at most 45,000, at most 40,000, at most 35,000, at most 30,000, at most 25,000, at most 20,000, at most 15,000, at most 10,000, at most 5,000, at most 1,000, at most 500, or at most 100 molecules per mm^2 . Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the surface density of hybridized target oligonucleotide molecules may range from about 5,000 molecules per mm^2 to about 50,000 molecules per mm^2 . Those of skill in the art will recognize that the surface density of hybridized target oligonucleotide molecules may have any value within this range, e.g., about 50,700 molecules per mm^2 .

In some instances, the target (or sample) oligonucleotide molecules (or nucleic acid molecules) hybridized to the oligonucleotide adapter or primer molecules attached to the low-binding support surface may range in length from about 0.02 kilobases (kb) to about 20 kb or from about 0.1 kilobases (kb) to about 20 kb. In some instances, the target oligonucleotide molecules may be at least 0.001 kb, at least 0.005 kb, at least 0.01 kb, at least 0.02 kb, at least 0.05 kb, at least 0.1 kb in length, at least 0.2 kb in length, at least 0.3 kb in length, at least 0.4 kb in length, at least 0.5 kb in length, at least 0.6 kb in length, at least 0.7 kb in length, at least 0.8 kb in length, at least 0.9 kb in length, at least 1 kb in length,

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at least 2 kb in length, at least 3 kb in length, at least 4 kb in length, at least 5 kb in length, at least 6 kb in length, at least 7 kb in length, at least 8 kb in length, at least 9 kb in length, at least 10 kb in length, at least 15 kb in length, at least 20 kb in length, at least 30 kb in length, or at least 40 kb in length, or any intermediate value spanned by the range described herein, e.g., at least 0.85 kb in length.

In some instances, the target (or sample) oligonucleotide molecules (or nucleic acid molecules) may comprise single-stranded or double-stranded, multimeric nucleic acid molecules (e.g., concatamers) further comprising repeats of a regularly occurring monomer unit. In some instances, the single-stranded or double-stranded, multimeric nucleic acid molecules may be at least 0.001 kb, at least 0.005 kb, at least 0.01 kb, at least 0.02 kb, at least 0.05 kb, at least 0.1 kb in length, at least 0.2 kb in length, at least 0.3 kb in length, at least 0.4 kb in length, at least 0.5 kb in length, at least 1 kb in length, at least 2 kb in length, at least 3 kb in length, at least 4 kb in length, at least 5 kb in length, at least 6 kb in length, at least 7 kb in length, at least 8 kb in length, at least 9 kb in length, at least 10 kb in length, at least 15 kb in length, or at least 20 kb in length, at least 30 kb in length, or at least 40 kb in length, or any intermediate value spanned by the range described herein, e.g., about 2.45 kb in length.

In some instances, the target (or sample) oligonucleotide molecules (or nucleic acid molecules) may comprise single-stranded or double-stranded multimeric nucleic acid molecules (e.g., concatamers) comprising from about 2 to about 100 copies of a regularly repeating monomer unit. In some instances, the number of copies of the regularly repeating monomer unit may be at least 2, at least 3, at least 4, at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, and at least 100. In some instances, the number of copies of the regularly repeating monomer unit may be at most 100, at most 95, at most 90, at most 85, at most 80, at most 75, at most 70, at most 65, at most 60, at most 55, at most 50, at most 45, at most 40, at most 35, at most 30, at most 25, at most 20, at most 15, at most 10, at most 5, at most 4, at most 3, or at most 2. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the number of copies of the regularly repeating monomer unit may range from about 4 to about 60. Those of skill in the art will recognize that the number of copies of the regularly repeating monomer unit may have any value within this range, e.g., about 17. Thus, in some instances, the surface density of hybridized target sequences in terms of the number of copies of a target sequence per unit area of the support surface may exceed the surface density of oligonucleotide primers even if the hybridization efficiency is less than 100%.

Nucleic Acid Surface Amplification (NASA):

As used herein, the phrase “nucleic acid surface amplification” (NASA) is used interchangeably with the phrase “solid-phase nucleic acid amplification” (or simply “solid-phase amplification”). In some aspects of the present disclosure, nucleic acid amplification formulations are described which, in combination with the disclosed hydrophilic, polymer-coated flow cell devices, provide for improved amplification rates, amplification specificity, and amplification efficiency. As used herein, specific amplification refers to amplification of template library oligonucleotide strands that have been tethered to the solid support either covalently or non-covalently. As used herein, non-

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specific amplification refers to amplification of primer-dimers or other non-template nucleic acids. As used herein, amplification efficiency is a measure of the percentage of tethered oligonucleotides on the support surface that are successfully amplified during a given amplification cycle or amplification reaction. Nucleic acid amplification performed on surfaces disclosed herein may obtain amplification efficiencies of at least 50%, 60%, 70%, 80%, 90%, 95%, or greater than 95%, such as 98% or 99%.

Any of a variety of thermal cycling or isothermal nucleic acid amplification schemes may be used with the disclosed low-binding supports. Examples of nucleic acid amplification methods that may be utilized with the disclosed low-binding supports include, but are not limited to, polymerase chain reaction (PCR), multiple displacement amplification (MDA), transcription-mediated amplification (TMA), nucleic acid sequence-based amplification (NASBA), strand displacement amplification (SDA), real-time SDA, bridge amplification, isothermal bridge amplification, rolling circle amplification, circle-to-circle amplification, helicase-dependent amplification, recombinase-dependent amplification, or single-stranded binding (SSB) protein-dependent amplification.

In some instances, improvements in amplification rate, amplification specificity, and amplification efficiency may be achieved using the disclosed hydrophilic, polymer-coated flow cell devices, either alone or in combination with formulations of the amplification reaction components. In addition to inclusion of nucleotides, one or more polymerases, helicases, single-stranded binding proteins, etc. (or any combination thereof), the amplification reaction mixture may be adjusted in a variety of ways to achieve improved performance including, but are not limited to, choice of buffer type, buffer pH, organic solvent mixtures, buffer viscosity, detergents and zwitterionic components, ionic strength (including adjustment of both monovalent and divalent ion concentrations), antioxidants and reducing agents, carbohydrates, BSA, polyethylene glycol, dextran sulfate, betaine, other additives, and the like.

The use of the disclosed hydrophilic, polymer-coated flow cell devices, alone or in combination with improved or optimized amplification reaction formulations, may yield increased amplification rates compared to those obtained using conventional supports and amplification protocols. In some instances, the relative amplification rates that may be achieved may be at least 2x, at least 3x, at least 4x, at least 5x, at least 6x, at least 7x, at least 8x, at least 9x, at least 10x, at least 12x, at least 14x, at least 16x, at least 18x, or at least 20x that for use of conventional supports and amplification protocols for any of the amplification methods described above.

In some instances, the use of the disclosed hydrophilic, polymer-coated flow cell devices, alone or in combination with improved or optimized buffer formulations, may yield total amplification reaction times (i.e., the time required to reach 90%, 95%, 98%, or 99% completion of the amplification reaction) of less than 180 mins, 120 mins, 90 min, 60 minutes, 50 minutes, 40 minutes, 30 minutes, 20 minutes, 15 minutes, 10 minutes, 5 minutes, 3 minutes, 1 minute, 50 s, 40 s, 30 s, 20 s, or 10 s for any of these completion metrics.

In some instances, the use of the disclosed low-binding supports alone or in combination with improved or optimized amplification buffer formulations may enable faster amplification reaction times (i.e., the times required to reach 90%, 95%, 98%, or 99% completion of the amplification reaction) of no more than 60 minutes, 50 minutes, 40 minutes, 30 minutes, 20 minutes, or 10 minutes. Similarly,

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use of the disclosed low-binding supports alone or in combination with improved or optimized buffer formulations may enable amplification reactions to be completed in some cases in no more than 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or no more than 30 cycles.

In some instances, the use of the disclosed hydrophilic, polymer-coated flow cell devices, alone or in combination with improved or optimized amplification reaction formulations, may yield increased specific amplification and/or decreased non-specific amplification compared to that obtained using conventional supports and amplification protocols. In some instances, the resulting ratio of specific amplification-to-non-specific amplification that may be achieved is at least 4:1 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 20:1, 30:1, 40:1, 50:1, 60:1, 70:1, 80:1, 90:1, 100:1, 200:1, 300:1, 400:1, 500:1, 600:1, 700:1, 800:1, 900:1, or 1,000:1.

In some instances, the use of the disclosed hydrophilic, polymer-coated flow cell devices, alone or in combination with improved or optimized amplification reaction formulations, may yield increased amplification efficiency compared to that obtained using conventional supports and amplification protocols. In some instances, the amplification efficiency that may be achieved is better than 50%, 60%, 70% 80%, 85%, 90%, 95%, 98%, or 99% in any of the amplification reaction times specified above.

In some instances, the clonally-amplified target (or sample) oligonucleotide molecules (or nucleic acid molecules) hybridized to the oligonucleotide adapter or primer molecules attached to the hydrophilic, polymer-coated flow cell device surfaces may range in length from about 0.02 kilobases (kb) to about 20 kb or from about 0.1 kilobases (kb) to about 20 kb. In some instances, the clonally-amplified target oligonucleotide molecules may be at least 0.001 kb, at least 0.005 kb, at least 0.01 kb, at least 0.02 kb, at least 0.05 kb, at least 0.1 kb in length, at least 0.2 kb in length, at least 0.3 kb in length, at least 0.4 kb in length, at least 0.5 kb in length, at least 1 kb in length, at least 2 kb in length, at least 3 kb in length, at least 4 kb in length, at least 5 kb in length, at least 6 kb in length, at least 7 kb in length, at least 8 kb in length, at least 9 kb in length, at least 10 kb in length, at least 15 kb in length, or at least 20 kb in length, or any intermediate value spanned by the range described herein, e.g., at least 0.85 kb in length.

In some instances, the clonally-amplified target (or sample) oligonucleotide molecules (or nucleic acid molecules) may comprise single-stranded or double-stranded, multimeric nucleic acid molecules (e.g., concatamers) further comprising repeats of a regularly occurring monomer unit. In some instances, the clonally-amplified single-stranded or double-stranded, multimeric nucleic acid molecules may be at least 0.1 kb in length, at least 0.2 kb in length, at least 0.3 kb in length, at least 0.4 kb in length, at least 0.5 kb in length, at least 1 kb in length, at least 2 kb in length, at least 3 kb in length, at least 4 kb in length, at least 5 kb in length, at least 6 kb in length, at least 7 kb in length, at least 8 kb in length, at least 9 kb in length, at least 10 kb in length, at least 15 kb in length, or at least 20 kb in length, or any intermediate value spanned by the range described herein, e.g., about 2.45 kb in length.

In some instances, the clonally-amplified target (or sample) oligonucleotide molecules (or nucleic acid molecules) may comprise single-stranded or double-stranded multimeric nucleic acid (e.g., concatamers) molecules comprising from about 2 to about 100 copies of a regularly repeating monomer unit. In some instances, the number of copies of the regularly repeating monomer unit may be at least 2, at least 3, at least 4, at least 5, at least 10, at least 15,

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at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, and at least 100. In some instances, the number of copies of the regularly repeating monomer unit may be at most 100, at most 95, at most 90, at most 85, at most 80, at most 75, at most 70, at most 65, at most 60, at most 55, at most 50, at most 45, at most 40, at most 35, at most 30, at most 25, at most 20, at most 15, at most 10, at most 5, at most 4, at most 3, or at most 2. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the number of copies of the regularly repeating monomer unit may range from about 4 to about 60. Those of skill in the art will recognize that the number of copies of the regularly repeating monomer unit may have any value within this range, e.g., about 12. Thus, in some instances, the surface density of clonally-amplified target sequences in terms of the number of copies of a target sequence per unit area of the support surface may exceed the surface density of oligonucleotide primers even if the hybridization and/or amplification efficiencies are less than 100%.

In some instances, the use of the disclosed hydrophilic, polymer-coated flow cell devices, alone or in combination with improved or optimized amplification reaction formulations, may yield increased clonal copy number compared to that obtained using conventional supports and amplification protocols. In some instances, e.g., wherein the clonally-amplified target (or sample) oligonucleotide molecules comprise concatenated, multimeric repeats of a monomeric target sequence, the clonal copy number may be substantially smaller than compared to that obtained using conventional supports and amplification protocols. Thus, in some instances, the clonal copy number may range from about 1 molecule to about 100,000 molecules (e.g., target sequence molecules) per amplified colony. In some instances, the clonal copy number may be at least 1, at least 5, at least 10, at least 50, at least 100, at least 500, at least 1,000, at least 2,000, at least 3,000, at least 4,000, at least 5,000, at least 6,000, at least 7,000, at least 8,000, at least 9,000, at least 10,000, at least 15,000, at least 20,000, at least 25,000, at least 30,000, at least 35,000, at least 40,000, at least 45,000, at least 50,000, at least 55,000, at least 60,000, at least 65,000, at least 70,000, at least 75,000, at least 80,000, at least 85,000, at least 90,000, at least 95,000, or at least 100,000 molecules per amplified colony. In some instances, the clonal copy number may be at most 100,000, at most 95,000, at most 90,000, at most 85,000, at most 80,000, at most 75,000, at most 70,000, at most 65,000, at most 60,000, at most 55,000, at most 50,000, at most 45,000, at most 40,000, at most 35,000, at most 30,000, at most 25,000, at most 20,000, at most 15,000, at most 10,000, at most 9,000, at most 8,000, at most 7,000, at most 6,000, at most 5,000, at most 4,000, at most 3,000, at most 2,000, at most 1,000, at most 500, at most 100, at most 50, at most 10, at most 5, or at most 1 molecule per amplified colony. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the clonal copy number may range from about 2,000 molecules to about 9,000 molecules. Those of skill in the art will recognize that the clonal copy number may have any value within this range, e.g., about 2,220 molecules in some instances, or about 2 molecules in others.

As noted above, in some instances the amplified target (or sample) oligonucleotide molecules (or nucleic acid mol-

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ecules) may comprise concatenated, multimeric repeats of a monomeric target sequence. In some instances, the amplified target (or sample) oligonucleotide molecules (or nucleic acid molecules) may comprise a plurality of molecules each of which comprises a single monomeric target sequence. Thus, the use of the disclosed hydrophilic, polymer-coated flow cell devices, alone or in combination with improved or optimized amplification reaction formulations, may result in a surface density of target sequence copies that ranges from about 100 target sequence copies per mm^2 to about 1×10^{12} target sequence copies per mm^2 . In some instances, the surface density of target sequence copies may be at least 100, at least 500, at least 1,000, at least 5,000, at least 10,000, at least 15,000, at least 20,000, at least 25,000, at least 30,000, at least 35,000, at least 40,000, at least 45,000, at least 50,000, at least 55,000, at least 60,000, at least 65,000, at least 70,000, at least 75,000, at least 80,000, at least 85,000, at least 90,000, at least 95,000, at least 100,000, at least 150,000, at least 200,000, at least 250,000, at least 300,000, at least 350,000, at least 400,000, at least 450,000, at least 500,000, at least 550,000, at least 600,000, at least 650,000, at least 700,000, at least 750,000, at least 800,000, at least 850,000, at least 900,000, at least 950,000, at least 1,000,000, at least 5,000,000, at least 1×10^7 , at least 5×10^7 , at least 1×10^8 , at least 5×10^8 , at least 1×10^9 , at least 5×10^9 , at least 1×10^{10} , at least 5×10^{10} , at least 1×10^{11} , at least 5×10^{11} , or at least 1×10^{12} of clonally amplified target sequence molecules per mm^2 . In some instances, the surface density of target sequence copies may be at most 1×10^{12} , at most 5×10^{11} , at most 1×10^{11} , at most 5×10^{10} , at most 1×10^{10} , at most 5×10^9 , at most 1×10^9 , at most 5×10^8 , at most 1×10^8 , at most 5×10^7 , at most 1×10^7 , at most 5,000,000, at most 1,000,000, at most 950,000, at most 900,000, at most 850,000, at most 800,000, at most 750,000, at most 700,000, at most 650,000, at most 600,000, at most 550,000, at most 500,000, at most 450,000, at most 400,000, at most 350,000, at most 300,000, at most 250,000, at most 200,000, at most 150,000, at most 100,000, at most 95,000, at most 90,000, at most 85,000, at most 80,000, at most 75,000, at most 70,000, at most 65,000, at most 60,000, at most 55,000, at most 50,000, at most 45,000, at most 40,000, at most 35,000, at most 30,000, at most 25,000, at most 20,000, at most 15,000, at most 10,000, at most 5,000, at most 1,000, at most 500, or at most 100 target sequence copies per mm^2 . Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the surface density of target sequence copies may range from about 1,000 target sequence copies per mm^2 to about 65,000 target sequence copies mm^2 . Those of skill in the art will recognize that the surface density of target sequence copies may have any value within this range, e.g., about 49,600 target sequence copies per mm^2 .

In some instances, the use of the disclosed low-binding supports alone or in combination with improved or optimized amplification buffer formulations may result in a surface density of clonally-amplified target (or sample) oligonucleotide molecules (or clusters) ranging from about from about 100 molecules per mm^2 to about 1×10^{12} colonies per mm^2 . In some instances, the surface density of clonally-amplified molecules may be at least 100, at least 500, at least 1,000, at least 5,000, at least 10,000, at least 15,000, at least 20,000, at least 25,000, at least 30,000, at least 35,000, at least 40,000, at least 45,000, at least 50,000, at least 55,000, at least 60,000, at least 65,000, at least 70,000, at least 75,000, at least 80,000, at least 85,000, at least 90,000, at least 95,000, at least 100,000, at least 150,000, at least

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200,000, at least 250,000, at least 300,000, at least 350,000, at least 400,000, at least 450,000, at least 500,000, at least 550,000, at least 600,000, at least 650,000, at least 700,000, at least 750,000, at least 800,000, at least 850,000, at least 900,000, at least 950,000, at least 1,000,000, at least 5,000, 5
000, at least 1×10^7 , at least 5×10^7 , at least 1×10^8 , at least 5×10^8 , at least 1×10^9 , at least 5×10^9 , at least 1×10^{10} , at least 5×10^{10} , at least 1×10^{11} , at least 5×10^{11} , or at least 1×10^{12} molecules per mm^2 . In some instances, the surface density of clonally-amplified molecules may be at most 1×10^{12} , at
10 most 5×10^{11} , at most 1×10^{11} , at most 5×10^{10} , at most 1×10^{10} , at most 5×10^9 , at most 1×10^9 , at most 5×10^8 , at most 1×10^8 , at most 5×10^7 , at most 1×10^7 , at most 5,000,000, at most 1,000,000, at most 950,000, at most 900,000, at most 850,000, at most 800,000, at most 750,000, at most 700,000, 15
at most 650,000, at most 600,000, at most 550,000, at most 500,000, at most 450,000, at most 400,000, at most 350,000, at most 300,000, at most 250,000, at most 200,000, at most 150,000, at most 100,000, at most 95,000, at most 90,000, at most 85,000, at most 80,000, at most 75,000, at most 70,000, 20
at most 65,000, at most 60,000, at most 55,000, at most 50,000, at most 45,000, at most 40,000, at most 35,000, at most 30,000, at most 25,000, at most 20,000, at most 15,000, at most 10,000, at most 5,000, at most 1,000, at most 500, or at most 100 molecules per mm^2 . Any of the lower and upper values described in this paragraph may be combined
25 to form a range included within the present disclosure, for example, in some instances the surface density of clonally-amplified molecules may range from about 5,000 molecules per mm^2 to about 50,000 molecules per mm^2 . Those of skill in the art will recognize that the surface density of clonally-amplified colonies may have any value within this range, e.g., about 48,800 molecules per mm^2 .

In some instances, the use of the disclosed hydrophilic, polymer-coated flow cell devices, alone or in combination with improved or optimized amplification reaction formulations, may yield signal from the amplified and labeled nucleic acid populations (e.g., a fluorescence signal) that has a coefficient of variance of no greater than 50%, such as 50%, 40%, 30%, 20%, 15%, 10%, 5%, or less than 5%. 35

Similarly, in some instances the use of the disclosed hydrophilic, polymer-coated flow cell devices, alone or in combination with improved or optimized amplification reaction formulations, may yield signal from the amplified and non-labeled nucleic acid populations that has a coefficient of variance of no greater than 50%, such as 50%, 40%, 30%, 20%, 10%, 5%, or less than 5%. 40

Fluorescence Imaging of Hydrophilic, Polymer-Coated Flow Cell Device Surfaces:

The disclosed hydrophilic, polymer-coated flow cell devices comprising, e.g., clonal clusters of labeled target nucleic acid molecules disposed thereon may be used in any of a variety of nucleic acid analysis applications, e.g., nucleic acid base discrimination, nucleic acid base classification, nucleic acid base calling, nucleic acid detection 55 applications, nucleic acid sequencing applications, and nucleic acid-based (genetic and genomic) diagnostic applications. In many of these applications, fluorescence imaging techniques may be used to monitor hybridization, amplification, and/or sequencing reactions performed on the low-binding supports. Fluorescence imaging may be performed using any of the optical imaging modules disclosed herein, as well as a variety of fluorophores, fluorescence imaging techniques, and other fluorescence imaging instruments known to those of skill in the art.

In some instances, the performance of nucleic acid hybridization and/or amplification reactions using the dis-

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closed hydrophilic, polymer-coated flow cell devices and reaction buffer formulations may be assessed using fluorescence imaging techniques, where the contrast-to-noise ratio (CNR) of the images provides a key metric in assessing amplification specificity and non-specific binding on the support. CNR is commonly defined as: $\text{CNR} = (\text{Signal} - \text{Background}) / \text{Noise}$. The background term is commonly taken to be the signal measured for the interstitial regions surrounding a particular feature (diffraction limited spot, DLS) in a specified region of interest (ROI). As noted above, while signal-to-noise ratio (SNR) is often considered to be a benchmark of overall signal quality, it can be shown that improved CNR can provide a significant advantage over SNR as a benchmark for signal quality in applications that
10 require rapid image capture (e.g., sequencing applications for which cycle times should be reduced or minimized). At high CNR, the imaging time required to reach accurate signal discrimination (and thus accurate base-calling in the case of sequencing applications) can be drastically reduced even with moderate improvements in CNR.

In most ensemble-based sequencing approaches, the background term is typically measured as the signal associated with ‘interstitial’ regions. In addition to “interstitial” background (B_{inter}), “intrastitial” background (B_{intra}) exists within the discrete regions occupied by amplified DNA colonies. The combination of these two background signal terms dictates the achievable CNR in the image, and subsequently directly impacts the optical instrument requirements, architecture costs, reagent costs, run-times, cost/genome, and ultimately the accuracy and data quality for cyclic array-based sequencing applications. The B_{inter} background signal arises from a variety of sources; a few examples include auto-fluorescence from consumable flow cells, non-specific adsorption of detection molecules that yield spurious fluorescence signals that may obscure the foreground signal from the ROI, and the presence of non-specific DNA amplification products (e.g., those arising from primer dimers). In typical next generation sequencing (NGS) applications, this background signal in the current field-of-view (FOV) is averaged over time and subtracted. The signal arising from individual DNA colonies (i.e., $(S) - B_{\text{inter}}$ in the FOV) yields a discernable feature that can be classified. In some instances, the intrastitial background (B_{intra}) can contribute a confounding fluorescence signal that is not specific to the target of interest but is present in the same ROI, thus making it far more difficult to average and subtract. 40

The implementation of nucleic acid amplification on the hydrophilic, polymer-coated substrate surfaces of the present disclosure may decrease the B_{inter} background signal by reducing non-specific binding, may lead to improvements in specific nucleic acid amplification, and may lead to a decrease in non-specific amplification that can impact the background signal arising from both the interstitial and intrastitial regions. In some instances, the disclosed low nonspecific binding support surfaces, optionally used in combination with improved hybridization and/or amplification reaction buffer formulations, may lead to improvements in CNR by a factor of 2, 5, 10, 100, 200, 500, or 1000-fold over those achieved using conventional supports and hybridization, amplification, and/or sequencing protocols. Although described here in the context of using fluorescence imaging as the read-out or detection mode, the same principles apply to the use of the disclosed low nonspecific binding supports and nucleic acid hybridization and amplification formulations for other detection modes as well, including both optical and non-optical detection modes. 65

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Alternative Sequencing Biochemistries:

In addition to the sequencing-by-nucleotide incorporation approach described above, the disclosed flow cell devices and optical imaging systems are compatible with other emerging nucleic acid sequencing biochemistries as well. Examples include the “sequencing-by-nucleotide binding” approach described in U.S. Pat. No. 10,655,176 B2, and the “sequencing-by-avidity” approach described in U.S. Pat. No. 10,768,173 B2.

The “sequencing-by-nucleotide binding” approach, as currently being developed by Omniome, Inc. (San Diego, Calif.) is based on performing repetitive cycles of detecting a stabilized complex that forms at each position along the template (e.g. a ternary complex that includes the primed template (tethered to a sample support structure), a polymerase, and a cognate nucleotide for the position), under conditions that prevent covalent incorporation of the cognate nucleotide into the primer, and then extending the primer to allow detection of the next position along the template. In the sequencing-by-binding approach, detection of the nucleotide at each position of the template occurs prior to extension of the primer to the next position. Generally, the methodology is used to distinguish the four different nucleotide types that can be present at positions along a nucleic acid template by uniquely labelling each type of ternary complex (i.e. different types of ternary complexes differing in the type of nucleotide it contains) or by separately delivering the reagents needed to form each type of ternary complex. In some instances, the labeling may comprise fluorescence labeling of, e.g., the cognate nucleotide or the polymerase that participate in the ternary complex. The approach is thus compatible with the disclosed flow cell devices and imaging systems.

The “sequencing-by-avidity” approach, as currently being developed by Element Biosciences, Inc. (San Diego, Calif.) relies on the increased avidity (or “functional affinity”) derived from forming a complex comprising a plurality of individual non-covalent binding interactions. Element’s approach is based on the detection of a multivalent binding complex formed between a fluorescently-labeled polymer-nucleotide conjugate, a polymerase, and a plurality of primed target nucleic acid molecules tethered to a sample support structure, which allows the detection/base-calling step to be separated from the nucleotide incorporation step. Fluorescence imaging is used to detect the bound complex and thereby determine the identity of the N+1 nucleotide in the target nucleic acid sequence (where the primer extension strand is N nucleotides in length). Following the imaging step, the multivalent binding complex is disrupted and washed away, the correct blocked nucleotide is incorporated into the primer extension strand, and the cycle is repeated.

In some instances, a polymer-nucleotide conjugate of the present disclosure may comprise a plurality of nucleotide moieties or nucleotide analog moieties conjugated to a polymer core, e.g., through the 5' end of the nucleotide, either directly or via a linker. By way of non-limiting example, the nucleotide moieties may include ribonucleotide moieties, ribonucleotide analog moieties, deoxyribonucleotide moieties, deoxyribonucleotide analog moieties, or any combination thereof. In some instances, the nucleotides or nucleotide analogs may comprise deoxyadenosine, deoxyguanosine, thymidine, deoxyuridine, deoxycytidine, adenosine, guanosine, 5-methyl-uridine, and/or cytidine. In some instances, the nucleotide or nucleotide analog moieties may comprise a nucleotide that has been modified to inhibit elongation during a polymerase reaction or a sequencing reaction, such as wherein the at least one nucleotide or

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nucleotide analog is a nucleotide that lacks a 3' hydroxyl group; a nucleotide that has been modified to contain a blocking group at the 3' position; and/or a nucleotide that has been modified with a 3'-O-azido group, a 3'-O-azidomethyl group, a 3'-O-alkyl hydroxylamino group, a 3'-phosphorothioate group, a 3'-O-malonyl group, or a 3'-O-benzyl group.

In some instances, the polymer core may comprise a linear or branched polymer, e.g., linear or branched polyethylene glycol (PEG), polypropylene glycol, polyvinyl alcohol, polylactic acid, polyglycolic acid, poly-glycine, polyvinyl acetate, a dextran, a protein, or other such polymers, or copolymers incorporating any two or more of the foregoing, or incorporating other polymers as are known in the art. In some instances, the polymer is a PEG. In some instances, the polymer is a branched PEG. In some instances, a branched polymer may comprise 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 or more branches or arms, or 2, 4, 8, 16, 32, 64, or more, branches or arms. In some instances, the branches or arms may radiate from a central moiety.

In some instances, the polymer-nucleotide conjugate may further comprise one or more detectable labels, e.g., one, two, three, four, five, six, seven, eight, nine, ten, fifteen, twenty, or more than twenty detectable labels. In some instances, the one or more detectable labels may comprise one or more fluorophores (e.g., cyanine dye 3 (Cy3), cyanine dye 5 (Cy5), etc.), one or more quantum dots, a fluorescence resonance energy transfer (FRET) donor, and/or a FRET acceptor.

In some instances, the polymer-nucleotide conjugate may further comprise a binding moiety attached to each branch of the polymer core or to a subset of branches. Examples of suitable binding moieties include, but are not limited to, biotin, avidin, streptavidin, or the like, polyhistidine domains, complementary paired nucleic acid domains, G-quartet forming nucleic acid domains, calmodulin, maltose-binding protein, cellulase, maltose, sucrose, glutathione-S-transferase, glutathione, 0-6-methylguanine-DNA methyltransferase, benzylguanine and derivatives thereof, benzylcysteine and derivatives thereof, an antibody, an epitope, a protein A, or a protein G. The binding moiety may be any interactive molecule or fragment thereof known in the art to bind to or facilitate interactions between proteins, between proteins and ligands, between proteins and nucleic acids, between nucleic acids, or between small molecule interaction domains or moieties.

As noted above, in the sequencing-by-avidity approach a multivalent binding complex is formed between, e.g., a fluorescently-labeled polymer-nucleotide conjugate, a polymerase, and a plurality of primed target nucleic acid molecules tethered to a sample support structure (e.g., a flow cell surface) when the nucleotide moieties of the polymer-nucleotide conjugate are complementary to a nucleotide residue of the target sequence. The stability of the multivalent binding complex thus formed allows the detection/base-calling step in a sequencing reaction cycle to be separated from the nucleotide incorporation step.

The stability of the multivalent binding complex—a ternary complex formed between two or more nucleotide moieties of the polymer-nucleotide conjugate, two or more polymerase molecules, and two or more primed target nucleic acid sequences—is evidenced by the prolonged persistence times of the complex. For example, in some instances, said multivalent binding complexes (ternary complexes) may have a persistence time of less than 0.5 seconds, less than 1 second, greater than 1 second, greater than 2 seconds, greater than 3 seconds, greater than 4 seconds,

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greater than 5 seconds, greater than 10 seconds, greater than 15 seconds, greater than 20 seconds, greater than 30 seconds, greater than 60 seconds, greater than 120 seconds, greater than 360 seconds, greater than 720 seconds, greater than 1,440 seconds, greater than 3,600 seconds, or more, or for a time within a range defined by any two or more of these values.

The use of polymer-nucleotide conjugates to form a multivalent binding complex with the polymerase and primed target nucleic acid results in an effective local concentration of the nucleotide that is increased many fold over the average nucleotide concentration that would be achieved using single unconjugated or untethered nucleotides, which in turn both enhances the stability of the complex and increases signal intensity following wash steps. The high signal intensity persists throughout the binding, washing, and imaging steps, and contributes to shorter image acquisition times. Following the imaging step, the multivalent binding complex can be destabilized, e.g., by changing the ionic composition, ionic strength, and/or the pH of the buffer, and washed away. A primer extension reaction may then be performed to extend the complementary strand by one base.

Nucleic Acid Sequencing System Performance:

In some instances, the disclosed nucleic acid sequencing systems, comprising one or more of the disclosed flow cell devices used in combination with one or more of the disclosed optical imaging systems, and optionally utilizing one of the emerging sequencing biochemistries such as the “sequencing-by-trapping” (or “sequencing-by-avidity”) approach described above, may provide improved nucleic acid sequencing performance in terms of, e.g., reduced sample input requirements, reduced image acquisition cycle time, reduced sequencing reaction cycle time, reduced sequencing run time, improved base-calling accuracy, reduced reagent consumption and cost, higher sequencing throughput, and reduced sequencing cost.

Nucleic Acid Sample Input (pM):

In some instances, the sample input requirements for the disclosed system may be significantly reduced due to the improved hybridization and amplification efficiencies that may be attained, and the high CNR images that may be acquired for base-calling, using the disclosed hydrophilic, polymer coated flow cell devices and imaging systems. In some instances, the nucleic acid sample input requirement for the disclosed systems may range from about 1 pM to about 10,000 pM. In some instances, the nucleic acid sample input requirement may be at least 1 pM, at least 2 pM, at least 5 pM, at least 10 pM, at least 20 pM, at least 50 pM, at least 100 pM, at least 200 pM, at least 500 pM, at least 1,000 pM, at least 2,000 pM, at least 5,000 pM, at least 10,000 pM. In some instances, the nucleic acid sample input requirement for the disclosed systems may be at most 10,000 pM, at most 5,000 pM, at most 2,000 pM, at most 1,000 pM, at most 500 pM, at most 200 pM, at most 100 pM, at most 50 pM, at most 20 pM, at most 10 pM, at most 5 pM, at most 2 pM, or at most 1 pM. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the nucleic acid sample input requirement for the disclosed systems may range from about 5 pM to about 500 pM. Those of skill in the art will recognize that the nucleic acid sample input requirement may have any value within this range, e.g., about 132 pM. In one exemplary instance, a nucleic acid sample input of about 100 pM is sufficient to generate signals for reliable base-calling.

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Nucleic Acid Sample Input (Nanograms):

In some instances, the nucleic acid sample input requirement for the disclosed systems may range from about 0.05 nanograms to about 1,000 nanograms. In some instances, the nucleic acid sample input requirement may be at least 0.05 nanograms, at least 0.1 nanograms, at least 0.2 nanograms, at least 0.4 nanograms, at least 0.6 nanograms, at least 0.8 nanograms, at least 1.0 nanograms, at least 2 nanograms, at least 4 nanograms, at least 6 nanograms, at least 8 nanograms, at least 10 nanograms, at least 20 nanograms, at least 40 nanograms, at least 60 nanograms, at least 80 nanograms, at least 100 nanograms, at least 200 nanograms, at least 400 nanograms, at least 600 nanograms, at least 800 nanograms, or at least 1,000 nanograms. In some instances, the nucleic acid sample input requirement may be at most 1,000 nanograms, at most 800 nanograms, at most 600 nanograms, at most 400 nanograms, at most 200 nanograms, at most 100 nanograms, at most 80 nanograms, at most 60 nanograms, at most 40 nanograms, at most 20 nanograms, at most 10 nanograms, at most 8 nanograms, at most 6 nanograms, at most 4 nanograms, at most 2 nanograms, at most 1 nanogram, at most 0.8 nanograms, at most 0.6 nanograms, at most 0.4 nanograms, at most 0.2 nanograms, at most 0.1 nanograms, or at most 0.05 nanograms. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the nucleic acid sample input requirement for the disclosed systems may range from about 0.6 nanograms to about 400 nanograms. Those of skill in the art will recognize that the nucleic acid sample input requirement may have any value within this range, e.g., about 2.65 nanograms.

#FOV Images Required to Tile Flow Cell:

In some instances, the field-of-view (FOV) of the disclosed optical imaging module is sufficiently large that a multi-channel (or multi-lane) flow cell (i.e., the fluid channel portions thereof) of the present disclosure may be imaged by tiling from about 10 FOV images (or “frames”) to about 1,000 FOV images (or “frames”). In some instances, an image of the entire multi-channel flow cell may require tiling at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 150, at least 200, at least 250, at least 300, at least 350, at least 400, at least 450, at least 500, at least 550, at least 600, at least 650, at least 700, at least 750, at least 800, at least 850, at least 900, at least 950, or at least 1,000 FOV images (or “frames”). In some instances, an image of the entire multi-channel flow cell may require tiling at most 1,000, at most 950, at most 900, at most 850, at most 800, at most 750, at most 700, at most 650, at most 600, at most 550, at most 500, at most 450, at most 400, at most 350, at most 300, at most 250, at most 200, at most 150, at most 100, at most 90, at most 80, at most 70, at most 60, at most 50, at most 40, at most 30, at most 20, or at most 10 FOV images (or “frames”). Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances an image of the entire multi-channel flow cell may require tiling from about 30 to about 100 FOV images. Those of skill in the art will recognize that in some instances the number of required FOV images may have any value within this range, e.g., about 54 FOV images.

Imaging Cycle Time:

In some instances, the combination of large FOV, image sensor response sensitivity, and/or fast FOV translation times enables shortened imaging cycle times (i.e., the time required to acquire a sufficient number of FOV images to tile the entire multichannel flow cell (or the fluid channel

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portions thereof). In some instances, the imaging cycle time may range from about 10 seconds to about 10 minutes. In some instances, the imaging cycle time may be at least 10 seconds at least 20 seconds, at least 30 seconds, at least 40 seconds, at least 50 seconds, at least 1 minute, at least 2 minutes, at least 3 minutes, at least 4 minutes, at least 5 minutes, at least 6 minutes, at least 7 minutes, at least 8 minutes, at least 9 minutes, or at least 10 minutes. In some instances, the imaging cycle time may be at most 10 minutes, at most 9 minutes, at most 8 minutes, at most 7 minutes, at most 6 minutes, at most 5 minutes, at most 4 minutes, at most 3 minutes, at most 2 minutes, at most 1 minute, at most 50 second, at most 40 second, at most 30 seconds, at most 20 seconds, or at most 10 seconds. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the imaging cycle time may range from about 20 seconds to about 1 minute. Those of skill in the art will recognize that in some instances the imaging cycle time may have any value within this range, e.g., about 57 seconds.

Sequencing Cycle Time:

In some instances, shortened sequencing reaction steps, e.g., due to reduced wash time requirements for the disclosed hydrophilic, polymer-coated flow cells, may result in shortened overall sequencing cycle times. In some instances, the sequencing cycle times for the disclosed systems may range from about 1 minute to about 60 minutes. In some instances, the sequencing cycle time may be at least 1 minute, at least 2 minutes, at least 3 minutes, at least 4 minutes, at least 5 minutes, at least 6 minutes, at least 7 minutes, at least 8 minutes, at least 9 minutes, at least 10 minutes, at least 15 minutes, at least 20 minutes, at least 25 minutes, at least 30 minutes, at least 35 minutes, at least 40 minutes, at least 45 minutes, at least 50 minutes, at least 55 minutes, or at least 60 minutes. In some instances, the sequencing reaction cycle time may be at most 60 minutes, at most 55 minutes, at most 50 minutes, at most 45 minutes, at most 40 minutes, at most 35 minutes, at most 30 minutes, at most 25 minutes, at most 20 minutes, at most 15 minutes, at most 10 minutes, at most 9 minutes, at most 8 minutes, at most 7 minutes, at most 6 minutes, at most 5 minutes, at most 4 minutes, at most 3 minutes, at most 2 minutes, or at most 1 minutes. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the sequencing cycle time may range from about 2 minutes to about 15 minutes. Those of skill in the art will recognize that in some instances the sequencing cycle time may have any value within this range, e.g., about 1 minute, 12 seconds.

Sequencing Read Length:

In some instances, the enhanced CNR images that may be achieved using the disclosed hydrophilic, polymer-coated flow cell devices in combination with the disclosed imaging systems, and in some cases, the use of milder sequencing biochemistries, may enable longer sequencing read lengths for the disclosed systems. In some instances, the maximum (single read) read length may range from about 50 bp to about 500 bp. In some instances, the maximum (single read) read length may be at least 50 bp, at least 100 bp, at least 150 bp, at least 200 bp, at least 250 bp, at least 300 bp, at least 350 bp, at least 400 bp, at least 450 bp, or at least 500 bp. In some instances, the maximum (single read) read length is at most 500 bp, at most 450 bp, at most 400 bp, at most 350 bp, at most 300 bp, at most 250 bp, at most 200 bp, at most 150 bp, at most 100 bp, or at most 50 bp. Any of the lower and upper values described in this paragraph may be com-

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bined to form a range included within the present disclosure, for example, in some instances the maximum (single read) read length may range from about 100 bp to about 450 bp. Those of skill in the art will recognize that in some instances the maximum (single read) read length may have any value within this range, e.g., about 380 bp.

Sequencing Run Time:

In some instances, the sequencing run time for the disclosed nucleic acid sequencing systems may range from about 8 hours to about 20 hours. In some instances, the sequencing run time is at least 8 hours, at least 9 hours, at least 10 hours, at least 12 hours, at least 14 hours, at least 16 hours, at least 18 hours, or at least 20 hours. In some instances, the sequencing run time is at most 20 hours, at most 18 hours, at most 16 hours, at most 14 hours, at most 12 hours, at most 10 hours, at most 9 hours, or at most 8 hours. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the sequencing run time may range from about 10 hours to about 16 hours. Those of skill in the art will recognize that in some instances the sequencing run time may have any value within this range, e.g., about 7 hours, 35 minutes.

Average Base-Calling Accuracy:

In some instances, the disclosed nucleic acid sequencing systems may provide an average base-calling accuracy of at least 80%, at least 85%, at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or at least 99.9% correct over the course of a sequencing run. In some instances, the disclosed nucleic acid sequencing systems may provide an average base-calling accuracy of at least 80%, at least 85%, at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or at least 99.9% correct per every 1,000 bases, 10,000 bases, 25,000 bases, 50,000 bases, 75,000 bases, or 100,000 bases called.

Average Q-Score:

In some instances, the quality or accuracy of a sequencing run may be assessed by calculating a Phred quality score (also referred to as a quality score or "Q-score"), which indicates the probability that a given base is called incorrectly by the sequencing system. For example, in some instances base calling accuracy for a specific sequencing chemistry and/or sequencing system may be assessed for a large empirical data set derived from performing sequencing runs on a library of known nucleic acid sequences. The Q-score may then be calculated according to the equation:

$$Q = -10 \log_{10} P$$

where P is the base calling error probability. A Q-score of 30, for example, indicates a probability of making a base calling error of 1 in every 1000 bases called (or a base calling accuracy of 99.9%).

In some instances, the disclosed nucleic acid sequencing systems may provide a more accurate base readout. In some instances, for example, the disclosed nucleic acid sequencing systems may provide a Q-score for base-calling accuracy over a sequencing run that ranges from about 20 to about 50. In some instances, the average Q-score for the run may be at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, or at least 50. Those of skill in the art will recognize that the average Q-score may have any value within this range, e.g., about 32.

Q-Score Vs. % Nucleotides Identified:

In some instances, the disclosed nucleic acid sequencing systems may provide a Q-score of greater than 20 for at least 50%, at least 60%, at least 70%, at least 80%, at least 85%,

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at least 90%, at least 95%, at least 98%, or at least 99% of the terminal (or N+1) nucleotides identified. In some instances, the disclosed nucleic acid sequencing systems may provide a Q-score of greater than 25 for at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% of the terminal (or N+1) nucleotides identified. In some instances, the disclosed nucleic acid sequencing systems may provide a Q-score of greater than 30 for at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% of the terminal (or N+1) nucleotides identified. In some instances, the disclosed nucleic acid sequencing systems may provide a Q-score of greater than 35 for at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% of the terminal (or N+1) nucleotides identified. In some instances, the disclosed nucleic acid sequencing systems may provide a Q-score of greater than 40 for at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% of the terminal (or N+1) nucleotides identified. In some instances, the disclosed nucleic acid sequencing systems may provide a Q-score of greater than 45 for at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% of the terminal (or N+1) nucleotides identified. In some instances, the disclosed compositions and methods for nucleic acid sequencing may provide a Q-score of greater than 50 for at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% of the terminal (or N+1) nucleotides identified.

Reagent Consumption:

In some instances, the disclosed nucleic acid sequencing systems may have lower reagent consumption rates and costs due to, e.g., the use of the disclosed flow cell devices and fluidic systems that minimize fluid channel volumes and dead volumes. In some instances, the disclosed nucleic acid sequencing systems may thus require an average of at least 5% less, at least 10% less, at least 15% less, at least 20% less, at least 25% less, at least 30% less, at least 35% less, at least 40% less, at least 45% less, or at least 50% less reagent by volume per Gbase sequenced than that consumed by an Illumina MiSeq sequencer.

Sequencing Throughput:

In some instances, the disclosed nucleic acid sequencing systems may provide a sequencing throughput ranging from about 50 Gbase/run to about 200 Gbase/run. In some

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instances, the sequencing throughput may be at least 50 Gbase/run, at least 75 Gbase/run, at least 100 Gbase/run, at least 125 Gbase/run, at least 150 Gbase/run, at least 175 Gbase/run, or at least 200 Gbase/run. In some instances, the sequencing throughput may be at most 200 Gbase/run, at most 175 Gbase/run, at most 150 Gbase/run, at most 125 Gbase/run, at most 100 Gbase/run, at most 75 Gbase/run, or at most 50 Gbase/run. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the sequencing throughput may range from about 75 Gbase/run to about 150 Gbase/run. Those of skill in the art will recognize that in some instances the sequencing throughput may have any value within this range, e.g., about 119 Gbase/run.

Sequencing Cost:

In some instances, the disclosed nucleic acid sequencing systems may provide nucleic acid sequencing at a cost ranging from about \$5 per Gbase to about \$30 per Gbase. In some instances, the sequencing cost may be at least \$5 per Gbase, at least \$10 per Gbase, at least \$15 per Gbase, at least \$20 per Gbase, at least \$25 per Gbase, or at least \$30 per Gbase. In some instances, the sequencing cost may be at most \$30 per Gbase, at most \$25 per Gbase, at most \$20 per Gbase, at most \$15 per Gbase, at most \$10 per Gbase, or at most \$30 per Gbase. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some instances the sequencing cost may range from about \$10 per Gbase to about \$15 per Gbase. Those of skill in the art will recognize that in some instances the sequencing cost may have any value within this range, e.g., about \$7.25 per Gbase.

EXAMPLES

These examples are provided for illustrative purposes only and not to limit the scope of the claims provided herein.

Example 1—Design Specifications for a Fluorescence Imaging Module for Genomics Applications

A non-limiting example of design specifications for a fluorescence imaging module of the present disclosure is provided in Table 1.

Table 1. Examples of design specifications for a fluorescence imaging module for genomics applications.

Design Parameter	Specification
Numerical aperture	≥0.3
Image quality	Diffraction limited
Field-of-view (FOV)	>2.0 mm ²
Image plane curvature	Best focal plane within 100 nm for >90% of the FOV, within 150 nm for 99% of the FOV, and within 200 nm for the entire FOV
Image distortion	<0.5% across the FOV
Magnification	2x to 20x
Camera pixel size at sample plane	≥2 x optical system modulation transfer function (MTF) limit
Coverslip thickness	>700 μm
Number of fluorescence imaging channels	≥3
Chromatic focal plane difference at camera between all imaging channels	≤100 nm equivalent at sample plane
Number of AF channels	1
Imaging time	≤2 seconds per FOV
Autofocus	Single step autofocus with error correction
Autofocus accuracy	<100 nm

Design Parameter	Specification
Scanning stage step and settle time	<0.4 seconds
Channel-specific optimized tube lens	1 per imaging channel
Illumination optical path	Liquid light guide with underfilled entrance aperture

Example 2—Fabrication of Glass Microfluidic Flow Cell Devices

Wafer-scale fabrication of microfluidic devices for use as flow cells can be constructed from, for example, one, two, or three layers of glass, e.g., borosilicate glass, fused-silica glass, or quartz, using one of the processes illustrated in FIGS. 36A-36C and a processing technique such as focused femtosecond laser photoablation and/or laser glass bonding.

In FIG. 36A, a first wafer is processed with a laser (e.g., that produces femtosecond laser radiation) to ablate the wafer material and provide a patterned surface. The patterned wafer surface may comprise a plurality of microfluidic devices (e.g., 12 devices per 210 mm diameter wafer), each of which may comprise a plurality of fluid channels. The processed wafer may then be diced to create individual microfluidic chips comprising open fluid channels that may optionally be subsequently sealed, e.g., by sealing with a film or by clamping the device to another support surface.

In FIG. 36B, a first wafer is processed to create a patterned surface which may then be placed in contact with and bonded to a second wafer to seal the fluid channels. Depending on the materials used, e.g., glass wafers, silicon wafers, etc., the bonding may be performed using, e.g., a thermal bonding process, an anodic bonding process, a laser glass bonding process, etc. The second wafer covers and/or seals the grooves, indentations, and/or apertures on the wafer having the patterned surface to form fluid channels and/or fluid chambers (e.g., the interior portion) of the device at the interface of the two wafer components. The bonded structure may then be diced into individual microfluidic chips, e.g., 12 microfluidic chips per 210 mm diameter wafer.

In FIG. 36C, the first wafer is processed to create a pattern of fluid channels that are cut or etched through the full thickness of the wafer (i.e., open on either surface of the wafer). The first wafer is then sandwiched between and bonded to a second wafer on one side and a third wafer on the other side. Depending on the materials used, e.g., glass wafers, silicon wafers, etc., the bonding may be performed using, e.g., a thermal bonding process, an anodic bonding process, a laser glass bonding process, etc. The second and third wafers cover and/or seal the grooves, indentations, and/or apertures in the first wafer to form fluid channels and/or fluid chambers (e.g., the interior portions) of the device. The bonded structure may then be diced into individual microfluidic chips, e.g., 12 microfluidic chips per 210 mm diameter wafer.

Example 3—Coating Flow Cell Surfaces with a Hydrophilic Polymer Coating

Glass flow cell devices were coated by washing prepared glass channels with KOH, followed by rinsing with ethanol and then silanization for 30 minutes at 65° C. Fluid channel surfaces were activated with EDC-NHS for 30 min., followed by grafting of oligonucleotide primers by incubation

of the activated surface with 5 μ m primer for 20 min., and then passivation with 30 μ m of an amino-terminated polyethylene glycol (PEG-NH₂).

Multilayer surfaces are made following the approach described above, where following the PEG-NH₂ passivation step, a multi-armed PEG-NETS is flowed through the fluid channels, followed by another addition of the PEG-NH₂, optionally followed by another incubation with PEG-NETS, and optionally followed by another incubation with multi-armed PEG-NH₂. For these surfaces, the primer may be grafted at any step, and especially following the last addition of multi-armed PEG-NH₂.

Example 4—Flow Cell Devices for Nucleic Acid Sequencing

FIG. 37A illustrates a non-limiting example of a one-piece glass microfluidic chip/flow cell design. In this design, fluid channels and inlet/outlet holes may be fabricated using, e.g., focused femtosecond laser radiation. There are two fluid channels (“lanes”) in the flow cell device, and each fluid channel comprises, e.g., 2 rows of 26 frames each (i.e., where a “frame” is the image area equivalent to the field-of-view for a corresponding imaging module) each, such that tiling 2×26=52 images suffices to image an entire fluid channel. The fluid channel can have, e.g., a depth of about 100 μ m. Fluid channel 1 has an inlet hole A1 and an outlet hole A2, and fluid channel 2 has an inlet hole B1 and an outlet hole B2. The flow cell device may also comprise a 1D linear, human-readable and/or machine-readable barcode, and optionally a 2D matrix barcode.

FIG. 37B illustrates a non-limiting example of a two-piece glass microfluidic chip/flow cell design. In this design, fluid channels and inlet/outlet holes may be fabricated using, e.g., focused femtosecond laser photoablation or photolithography and chemical etching processes. The 2 pieces can be bonded together using any of a variety of techniques as described above. The inlet and outlet holes may be positioned on the top layer of the structure and oriented in such a way that they are in fluid communication with at least one of the fluid channels and/or fluid chambers formed in the interior portion of the device. There are two fluid channels in the flow cell device, and as with the device illustrated in FIG. 37A, each fluid channel comprises, e.g., 2 rows with 26 frames in each row. The fluid channels can have, e.g., a depth of about 100 μ m. Fluid channel 1 has an inlet hole A1 and an outlet hole A2, and fluid channel 2 has an inlet hole B1 and an outlet hole B2. The flow cell device may also comprise a 1D linear, human-readable and/or machine-readable barcode, and optionally a 2D matrix barcode.

FIG. 37C illustrates a non-limiting example of a three-piece glass microfluidic chip/flow cell design. In this design, fluid channels and inlet/outlet holes may be fabricated using, e.g., focused femtosecond laser photoablation or photolithography and chemical etching processes. The 3 pieces can be bonded together using any of a variety of techniques as described above. The first wafer (comprising a through-

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pattern of fluid channels or fluid chambers) can be sandwiched between and bonded to a second wafer on one side and a third wafer on the other side. The inlet and outlet holes may be positioned on the top layer of the structure and oriented in a way such that they are in fluid communication with at least one of the fluid channels and/or fluid chambers formed in the interior portion of the device. There are two fluid channels in the flow cell device, and as with the devices illustrated in FIGS. 37A and 37B, each fluid channel has 2 rows with 26 frames in each row. The fluid channel can have a depth of, e.g., about 100 μm . Fluid channel 1 has an inlet hole A1 and an outlet hole A2, and fluid channel 2 has an inlet hole B1 and an outlet hole B2. The flow cell device may also comprise a 1D linear, human-readable and/or machine-readable barcode, and optionally a 2D matrix barcode.

Example 5—Imaging of Nucleic Acid Clusters in a Capillary Flow Cell

Nucleic acid clusters were established within a capillary and subjected to fluorescence imaging. A flow device having a capillary tube was used for the test. An example of the resulting cluster images is presented in FIG. 38. The figure demonstrated that nucleic acid clusters formed by amplification within the lumen of a capillary flow cell device as disclosed herein can be reliably formed and visualized.

Example 6—Plastic Sample Support Structures

In some instances, the disclosed samples support structures may be fabricated from a polymer. Examples of materials from which the sample support structure, e.g., a capillary flow cell device, may be fabricated include, but are not limited to, polystyrene (PS), macroporous polystyrene (MPPS), polymethylmethacrylate (PMMA), polycarbonate (PC), polypropylene (PP), polyethylene (PE), high density polyethylene (HDPE), cyclic olefin polymers (COP), cyclic olefin copolymers (COC), polyethylene terephthalate (PET), or any combination thereof. Various compositions comprising both glass and plastic substrates are also contemplated.

Modification of a polymer surface for the surface coating purposes disclosed herein involves making surfaces reactive with other chemical groups ($-\text{R}$), including amines. When prepared on an appropriate substrate, these reactive surfaces can be stored long term at room temperature, for example, for at least 3 months or more in some instances. Such surfaces can be further grafted with R-PEG and R-primer oligomer for on-surface amplification of nucleic acids, as described elsewhere herein. Plastic surfaces, such as cyclic olefin polymer (COP), may be modified using any of a variety of methods known in the art. For example, they can be treated with Ti:Sapphire laser ablation, UV-mediated ethylene glycol methacrylate photografting, plasma treatment, or mechanical agitation (e.g., sand blasting, or polishing, etc.) to create hydrophilic surfaces that can remain reactive for months towards a variety of chemical groups, such as amines. These groups may then allow conjugation of passivation polymers such as PEG, or biomolecules such as DNA or proteins, without loss of biochemical activity. For example, attachment of DNA primer oligomers allows DNA amplification on a passivated plastic surface while reducing or minimizing the non-specific adsorption of proteins, fluorophore molecules, or other hydrophobic molecules.

Additionally, in some instances, surface modification can be combined with, e.g., laser printing or UV masking, to create patterned surfaces. This allows patterned attachment of DNA oligomers, proteins, or other moieties, providing for

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surface-based enzymatic activity, binding, detection, or processing. For example, DNA oligomers may be used to amplify DNA only within patterned features, or to capture amplified long DNA concatemers in a patterned fashion. In some embodiments, enzyme islands may be generated in the patterned areas that are capable of reacting with solution-based substrates. Because plastic surfaces are especially amenable to these processing modes, in some embodiments as contemplated herein, plastic sample support surfaces or flow cell devices may be recognized as being particularly advantageous.

Furthermore, plastic can be injection molded, embossed, stamped, or 3D printed to form any shape, including microfluidic devices, much more easily than glass substrates, and thus can be used to create surfaces for the binding and analysis of biological samples in multiple configurations, e.g., sample-to-result microfluidic chips for biomarker detection or DNA sequencing.

Specific and localized DNA amplification on modified plastic surfaces can be performed to produce nucleic acid spots with an ultra-high contrast to noise ratio and very low background when probed with fluorescent labels.

Hydrophilized and amine-reactive cyclic olefin polymer surface with amine-primer and amine-PEG can be prepared and has been demonstrated to support rolling circle amplification. When probed with fluorophore labeled primers, or when labeled dNTPs were added to the hybridized primers by a polymerase, bright spots of DNA amplicons were observed that exhibited signal to noise ratios greater than 100 with backgrounds that are extremely low, indicating highly specific amplification, and ultra-low levels of non-specific protein and hydrophobic fluorophore binding, which are hallmarks of the high accuracy detection required for systems such as fluorescence-based DNA sequencers.

Example 7—Prophetic Example of the Use of a Structured Illumination Imaging System for Sequencing

A structured illumination imaging system 4100 such as the non-limiting example illustrated in FIG. 41 may be used in combination with a flow cell 4187 comprising a low non-specific binding surface to perform nucleic acid sequencing. Target nucleic acid sequences are hybridized to adapter/primer sequences attached to the low non-specific binding surface 4188 on the interior of the flow cell 4187 at high surface density and clonally amplified using hybridization and amplification buffers that are specially formulated for said surface to enhance specific hybridization and amplification rates.

The flow cell 4187 is mounted in the structured illumination imaging system 4100, and a sequencing reaction cycle comprising the use of, e.g., the polymer-nucleotide conjugate chemistry described above and the workflow illustrated in FIG. 40 is initiated. The fluorescently labeled polymer nucleotide conjugate is introduced into the flow cell 4187 and contacted with the surface 4188 to form multivalent binding complexes if the nucleotide moiety of the polymer-nucleotide conjugate is complementary to a nucleotide of the target sequence. Excess, unbound polymer-nucleotide conjugate is then rinsed away.

For each detection step, a series of images of surface 4188 are captured using different orientations of a diffraction grating, e.g., 4130A, in at least one branch of an illumination optical path and at several different positions of an optical phase modulator, e.g., 4140A, to project illumination light fringe patterns onto the surface 4188. Following image

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acquisition, the series of images are processed using an image reconstruction algorithm to generate a higher resolution image than that achievable using diffraction-limited optics alone. The process may be repeated for several positions on surface **4188** to create a tiled image of the interior flow cell surface. Optionally, the focal plane may be adjusted, and the process may be repeated to generate higher resolution images of a second interior flow cell surface **4189**.

The combination of high contrast-to-noise ratio images (achieved using the disclosed low-binding surfaces with multiply-labeled polymer-nucleotide conjugate sequencing chemistry) and efficient processing of a relatively small number of images acquired using a structured illumination imaging system to image flow cell surfaces at super-resolution (thus enabling the use of higher surface densities of target sequence clusters) may contribute to higher overall sequencing throughput.

Example 8—Prophetic Example of Using a Multiplexed Read-Head for Dual Surface Imaging

A multiplexed read-head such as that illustrated schematically in FIGS. **44A** and **44B** is designed to perform dual surface imaging. The read-head comprises a plurality of microfluorometers which are assembled so that they are held in a fixed positions relative to one another and may be scanned in a direction horizontal to a pair of opposed interior flow cell surfaces to acquire images of a swath of each surface. As illustrated in FIG. **44A**, a first subset of the plurality of microfluorometers is configured to acquire images of a first interior flow cell surface, and a second subset of the plurality of microfluorometers is configured to acquire images of a second interior flow cell surface that faces the first interior surface and is separated from it by the thickness of an intervening fluid channel.

A flow cell comprising a low non-specific binding surface coating is used to perform nucleic acid sequencing. Target nucleic acid sequences are hybridized to adapter/primer sequences attached to the low non-specific binding surfaces on the interior of the flow cell and clonally amplified using hybridization and amplification buffers that are specially formulated for said surfaces to enhance specific hybridization and amplification rates.

The flow cell is mounted in an imaging system comprising the multiplexed read-head, and a sequencing reaction cycle comprising the use of, e.g., the polymer-nucleotide conjugate chemistry described above and the workflow illustrated in FIG. **40** is initiated. The fluorescently labeled polymer nucleotide conjugate is introduced into the flow cell and contacted with the interior surfaces to form multivalent binding complexes if the nucleotide moiety of the polymer-nucleotide conjugate is complementary to a nucleotide of the target sequence. Excess, unbound polymer-nucleotide conjugate is then rinsed away.

For each detection step, the multiplexed read-head is scanned in at least one direction parallel to the interior surfaces of the flow cell (or the flow cell may be scanned relative to the multiplexed read-head) and images of both the first and second interior flow cell surfaces are acquired simultaneously, as illustrated in FIG. **44B**, while an autofocus mechanism maintains the proper working distance between the objectives of the multiplexed read-head and at least one of the interior flow cell surfaces.

The ability to image both flow cell surfaces simultaneously using a single-pass scan of the flow cell (depending on

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the design of the read-head) may provide significant improvements in sequencing throughput.

Additional Numbered Embodiments

1. A system for sequencing nucleic acid molecules comprising:
 - a) a flow cell with an interior surface comprising a plurality of primed target nucleic acid sequences coupled thereto, wherein a primed target nucleic acid sequence of the plurality of primed target nucleic acid sequences has a polymerase bound thereto;
 - b) a fluid flow controller configured to control sequential and iterative delivery of a reagent to the interior surface of the flow cell;
 - c) an imaging module comprising:
 - i) a structured illumination system; and
 - ii) an image acquisition system configured to acquire images of the interior surface of the flow cell; and
 - d) a processor, wherein the processor is programed to instruct the system to perform an iterative method comprising:
 - i) contacting the plurality of primed target nucleic acid sequences coupled to the interior surface of the flow cell with a nucleotide composition to form a transient binding complex between the plurality of primed target nucleic acid sequences and a plurality of nucleotide moieties when a nucleotide moiety of the nucleotide composition is complementary to a nucleotide of the primed target nucleic acid sequence; and
 - ii) imaging the interior surface of the flow cell to detect the transient binding complex and thereby determine an identity of the nucleotide of the primed target nucleic acid sequence.
2. The system of claim 1, wherein the structured illumination system comprises an optical system designed to project periodic patterns of light on the interior surface of the flow cell, and wherein a relative orientation or phase shift of a plurality of the periodic patterns of light may be changed in a known manner.
3. The system of claim 1, wherein the structured illumination system comprises a first optical arm comprising a first light emitter to emit light and a first beam splitter to split light emitted by the first light emitter to project a first plurality of fringes on the interior surface of the flow cell.
4. The system of claim 3, wherein the structured illumination system further comprises a second optical arm comprising a second light emitter to emit light and a second beam splitter to split light emitted by the second light emitter to project a second plurality of fringes on the interior surface of the flow cell.
5. The system of claim 4, wherein the structured illumination system further comprises an optical element to combine an optical path of the first arm and the second arm.
6. The system of claim 4 or claim 5, wherein the first beam splitter comprises a first transmissive diffraction grating and the second beam splitter comprises a second transmissive diffraction grating.
7. The system of claim 4 or claim 5, wherein the first and second light emitters emit unpolarized light, and wherein the first and second transmissive diffraction gratings are to diffract unpolarized light emitted by a respective one of the first and second light emitters.
8. The system of claim 6 or claim 7, wherein the optical element to combine an optical path of the first plurality of fringes and the second plurality of fringes comprises a

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mirror with holes, with the mirror arranged to reflect light diffracted by the first transmissive diffraction grating and with the holes arranged to pass through at least first orders of light diffracted by the second transmissive diffraction grating.

9. The system of claim 8, further comprising: one or more optical elements to phase shift the first plurality of fringes and the second plurality of fringes.

10. The system of claim 9, wherein the one or more optical elements to phase shift the first plurality of fringes and the second plurality of fringes comprise a first rotating optical window to phase shift the first plurality of fringes and a second rotating optical window to phase shift the second plurality of optical fringes.

11. The system of claim 9 or claim 10, wherein the one or more optical elements to phase shift the first plurality of fringes and the second plurality of fringes comprise a first linear motion stage to translate the first diffraction grating and a second linear motion stage to translate the second diffraction grating.

12. The system of any one of claims 9 to 11, wherein the one or more optical elements to phase shift the first plurality of fringes and the second plurality of fringes comprise a single rotating optical window, wherein the single rotating optical window is positioned after the mirror with holes in an optical path to the sample.

13. The system of claim 12, wherein an axis of rotation of the single rotating optical window is offset by about 45 degrees from an optical axis of each of the gratings.

14. The system of any one of claims 9 to 13, wherein the first plurality of fringes are angularly offset from the second plurality of fringes on the sample plane by about 90 degrees.

15. The system of claim 14, wherein the sample comprises a plurality of features regularly patterned in a rectangular array or hexagonal array.

16. The system of any one of claims 9 to 15, further comprising: an objective lens to project each of the first plurality of fringes and the second plurality of fringes on the sample.

17. The system of any one of claims 9 to 16, further comprising: one or more optical beam blockers for blocking zero orders of light emitted by each of the first and second diffraction gratings.

18. The system of claim 17, wherein the one or more optical beam blockers comprise a Bragg grating.

19. The system of any one of claims 6 to 18, wherein the optical element to combine an optical path of the first arm and the second arm comprises a polarizing beam splitter, wherein the first diffraction grating diffracts vertically polarized light and wherein the second diffraction grating diffracts horizontally polarized light.

20. The system of any one of claims 4 to 19, wherein the first and second beam splitters each comprise a beam splitter cube or plate.

21. The system of any one of claims 3 to 20, wherein the first beam splitter comprises a first reflective diffraction grating and the second beam splitter comprises a second reflective diffraction grating.

22. The system of any one of claims 1 to 21, wherein the structured illumination system comprises a multiple beam splitter slide comprising a plurality of beam splitters mounted on a linear translation stage such that the plurality of beam splitters have fixed orientations with respect to the system's optical axis.

23. The system of claim 22, wherein the plurality of beam splitters comprises a plurality of diffraction gratings.

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24. The system of claim 23, wherein the plurality of diffraction gratings comprises two diffraction gratings.

25. The system of any one of claims 1 to 24, wherein the structured illumination system comprises a fixed two-dimensional diffraction grating used in combination with a spatial filter wheel to project one-dimensional diffraction patterns on the interior surface of the flow cell.

26. The system of any one of claims 1 to 25, wherein the image acquisition system comprises a custom tube lens which, in combination with an objective, enables imaging of a first interior flow cell surface and a second interior flow cell surface with substantially the same image resolution.

27. The system of any one of claims 1 to 26, wherein the nucleotide composition comprises a conjugated polymer-nucleotide composition.

28. The system of claim 27, wherein the conjugated polymer-nucleotide composition comprises a plurality of nucleotide moieties conjugated to a polymer core.

29. The system of claim 28, wherein the plurality of nucleotide moieties comprises nucleotides, nucleotide analogs, or any combination thereof.

30. The system of claim 28 or claim 29, wherein the plurality of nucleotide moieties comprises a plurality of identical nucleotide moieties.

31. The system of any one of claims 1 to 30, wherein prior to forming the transient binding complex the nucleotide composition lacks a polymerase.

32. A method for sequencing nucleic acid molecules comprising:

a) providing a plurality of primed target nucleic acid sequences tethered to a surface, wherein a primed target nucleic acid sequence of the plurality of primed target nucleic acid sequences has a polymerase bound thereto;

b) contacting the plurality of primed target nucleic acid sequences with a nucleotide composition to form a transient binding complex between the plurality of primed target nucleic acid sequences and a plurality of nucleotide moieties when a nucleotide moiety of the nucleotide composition is complementary to a nucleotide of the primed target nucleic acid sequence; and

c) detecting the transient binding complex to determine the identity of the nucleotide of the primed target nucleic acid sequence, wherein the detecting comprises:

i) illuminating the surface with light provided by a structured illumination system under a first set of illumination conditions to project a first plurality of fringes oriented in a specific direction on the surface;

ii) capturing a first plurality of phase images of the surface, wherein during capture of the first plurality of images, the positions of the first plurality of fringes are shifted on the surface;

iii) illuminating the surface with light provided by the structured illumination system under a second set of illumination conditions to project a second plurality of fringes on the surface, wherein the second plurality of fringes are angularly offset from the first plurality of fringes on the surface; and

iv) capturing a second plurality of phase images of the surface illuminated with the second plurality of fringes, wherein during capture of the second plurality of fringes, the positions of the second plurality of fringes are shifted on the surface.

33. The method of claim 32, wherein the structured illumination system comprises a first optical arm comprising a first light emitter to emit light and a first diffraction grating to

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diffract light emitted by the first light emitter to project the first plurality of fringes oriented in a specific direction on the surface.

34. The method of claim 33, wherein the structured illumination system comprises a second optical arm comprising a second light emitter to emit light and a second diffraction grating to diffract light emitted by the second light emitter to project the second plurality of fringes that are angularly offset from the first plurality of fringes on the surface.

35. The method of any one of claims 32 to 34, wherein the structured illumination system comprises a multiple beam splitter slide comprising a plurality of beam splitters mounted on a linear translation stage such that the plurality of beam splitters have fixed orientations with respect to the system's optical axis, and wherein the first set of illumination conditions corresponds to a first position of the linear translation stage and the second set of illumination conditions corresponds to a second position of the linear translation stage.

36. The method of claim 35, wherein the plurality of beam splitters comprises a plurality of diffraction gratings.

37. The method of claim 36, wherein the plurality of diffraction gratings comprises two diffraction gratings.

38. The method of any one of claims 32 to 37, wherein the structured illumination system comprises a fixed two-dimensional diffraction grating used in combination with a spatial filter wheel to project one-dimensional diffraction patterns on the surface, and wherein the first set of illumination conditions corresponds to a first position of the spatial filter wheel and the second set of illumination conditions corresponds to a second position of the spatial filter wheel.

39. The method of any one of claims 34 to 38, wherein the first diffraction grating and the second diffraction grating are transmissive diffraction gratings, wherein the structured illumination system comprises a mirror with holes to reflect light diffracted by the first diffraction grating and to pass through at least first orders of light diffracted by the second diffraction grating.

40. The method of any one of claims 32 to 39, further comprising: using at least the first plurality of captured phase images and the second plurality of captured phased images to computationally reconstruct one or more images having higher resolution than each of the first and second pluralities of captured phased images.

41. The method of claim 40, wherein the first plurality of fringes is angularly offset from the second plurality of fringes on the surface by about 90 degrees.

42. The method of any one of claims 32 to 41, wherein the surface comprises a plurality of features regularly patterned in a rectangular array or hexagonal array.

43. The method of any one of claims 32 to 42, wherein the first plurality of fringes and the second plurality of fringes are phase shifted by rotating a single optical window positioned in an optical path between the surface and each of the first and second diffraction gratings, wherein an axis of rotation of the single rotating optical window is offset from an optical axis of each of the diffraction gratings.

44. The method of any one of claims 34 to 43, wherein the first optical arm is turned off and the second optical arm of the structured illumination system is turned on after capturing the first plurality of phase images.

45. The method of any one of claims 34 to 44, wherein the first diffraction grating and the second diffraction grating are mechanically fixed during image capture.

46. The method of any one of claims 32 to 45, wherein the nucleotide composition comprises a conjugated polymer-nucleotide composition.

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47. The method of claim 46, wherein the conjugated polymer-nucleotide composition comprises a plurality of nucleotide moieties conjugated to a polymer core.

48. The method of claim 47, wherein the plurality of nucleotide moieties comprises nucleotides, nucleotide analogs, or any combination thereof.

49. The method of claim 47 or claim 48, wherein the plurality of nucleotide moieties comprises a plurality of identical nucleotide moieties.

50. The method of any one of claims 32 to 49, wherein the method is used to determine the identity of an N+1 or terminal nucleotide of a primer strand of the primed target nucleic acid sequence.

51. The method of any one of claims 32 to 50, wherein prior to forming the transient binding complex the nucleotide composition lacks a polymerase.

52. A detection apparatus, comprising

a) a read-head assembly comprising a plurality of microfluorometers,

wherein the plurality of microfluorometers are held in fixed positions relative to each other to form a multiplexed read-head,

wherein at least one of a first subset of the plurality of microfluorometers is configured to acquire a wide-field image of a different area of a first sample plane, and

wherein at least one of a second subset of the plurality of microfluorometers is configured to acquire a wide-field images of a different area of a second sample plane.

53. The detection apparatus of claim 52, further comprising a translation stage configured to move the read-head assembly in at least one direction parallel to the first and second sample planes.

54. The detection apparatus of claim 52 or claim 53, further comprising a sample stage configured to hold a flow cell comprising first and second interior surfaces such that the first interior surface is held at the first sample plane, and the second interior surface is held at the second sample plane.

55. The detection apparatus of any one of claims 52 to 54, wherein at least one microfluorimeter of the plurality of microfluorimeters is configured to acquire wide-field images having a field-of-view of at least 1 mm.

56. The detection apparatus of any one of claims 52 to 55, wherein at least one microfluorimeter of the plurality of microfluorimeters is configured to acquire wide-field images having a field-of-view of at least 1.5 mm.

57. The detection apparatus of any one of claims 52 to 56, wherein at last one of the microfluorometers further comprises a dedicated autofocus mechanism.

58. The detection apparatus of claim 57, wherein the autofocus mechanism for a first microfluorometer is configured to integrate data from an autofocus mechanism for a second microfluorometer, whereby the autofocus mechanisms for the first microfluorometer alters a focus of the first microfluorometer based on a focus position of the first microfluorometer and a focus position of the second microfluorometer.

59. The detection apparatus of any one of claims 52 to 58, wherein an individual microfluorometer further comprises an objective, a beam splitter and a detector, wherein the beam splitter is positioned to direct excitation radiation from an excitation radiation source to the objective and to direct emission radiation from the objective to the detector.

60. The detection apparatus of claim 59, wherein at least one individual microfluorometer further comprises an individual excitation radiation source.

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61. The detection apparatus of claim **59** or claim **60**, wherein the excitation radiation source directs the excitation radiation to the objectives of two or more individual microfluorometers of the plurality such that the two or more individual microfluorometers share the excitation radiation source.

62. The detection apparatus of any one of claims **59** to **61**, wherein two or more individual microfluorometers of the plurality further comprise or share at least two excitation radiation sources.

63. The detection apparatus of any one of claims **59** to **62**, wherein the objectives of the individual microfluorometers of the plurality have a numerical aperture between 0.2 and 0.5.

64. The detection apparatus of any one of claims **52** to **63**, wherein the microfluorometers of the plurality are configured to acquire images at a resolution sufficient to distinguish features that are less than 50 microns apart.

65. The detection apparatus of any one of claims **52** to **64**, wherein the microfluorometers of the plurality are configured to have a depth-of-field that is less than the separation distance between the first and second interior surfaces of the flow cell.

66. The detection apparatus of any one of claims **52** to **65**, wherein the first subset of the plurality of microfluorometers is configured to acquire wide-field images at a first fluorescence emission wavelength and the second subset of the plurality of microfluorometers is configured to acquire wide field images at a second fluorescence emission wavelength.

67. A method for determining an identity of a nucleotide in a target nucleic acid sequence comprising:

a) providing a plurality of primed target nucleic acid sequences, wherein a primed target nucleic acid sequence of the plurality of primed target nucleic acid sequences has a polymerase bound thereto;

b) contacting the plurality of primed target nucleic acid sequences with a nucleotide composition to form a transient binding complex between the plurality of primed target nucleic acid sequences and a plurality of nucleotide moieties when a nucleotide moiety of the nucleotide composition is complementary to a nucleotide of the primed target nucleic acid sequence; and

c) detecting the transient binding complex to determine the identity of the nucleotide of the primed target nucleic acid sequence, wherein the detecting comprises:

translating a multiplexed read-head in at least one direction parallel to a surface on which the plurality of primed target nucleic acid sequences is tethered, wherein the multiplexed read-head comprises a plurality of microfluorometers held in fixed positions relative to each other, and

wherein at least one microfluorimeter of the plurality of microfluorometers is configured to acquire a wide-field image of a different area of the surface than other microfluorimeters of the plurality.

68. The method of claim **67**, wherein the nucleotide composition comprises a conjugated polymer-nucleotide composition.

69. The method of claim **68**, wherein the conjugated polymer-nucleotide composition comprises a plurality of nucleotide moieties conjugated to a polymer core.

70. The method of claim **69**, wherein the plurality of nucleotide moieties comprises nucleotides, nucleotide analogs, or any combination thereof.

71. The method of claim **69** or claim **70**, wherein the plurality of nucleotide moieties comprises a plurality of identical nucleotide moieties.

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72. The method of any one of claims **67** to **71**, wherein the method is used to determine the identity of an N+1 or terminal nucleotide of a primer strand of the primed target nucleic acid sequence.

73. The method of any one of claims **67** to **72**, wherein prior to forming the transient binding complex the nucleotide composition lacks a polymerase.

74. The method of any one of claims **67** to **73**, wherein the plurality of primed target nucleic acid sequences is tethered to a first interior surface and a second interior surface of a flow cell, and wherein a first subset of the plurality of microfluorometers is configured to acquire wide-field images of different areas of the first interior surface of the flow cell, and a second subset of the plurality of microfluorometers is configured to acquire wide-field images of different areas of the second interior surface of the flow cell.

75. A system for sequencing nucleic acid molecules comprising:

a) a flow cell having at least one interior surface comprising a plurality of primed target nucleic acid sequences coupled thereto, wherein a primed target nucleic acid sequence of the plurality of primed target nucleic acid sequences has a polymerase bound thereto;

b) a fluid flow controller configured to control sequential and iterative delivery of a reagent to the at least one interior surface of the flow cell;

c) an imaging module configured to image the at least one interior surface of the flow cell, wherein the imaging module comprises:

a multiplexed read-head assembly comprising a plurality of microfluorometers held in fixed positions relative to each other,

wherein at least one microfluorimeter of the plurality of microfluorometers is configured to acquire a wide-field image of a different area of the at least one surface than other microfluorimeters of the plurality; and

d) a processor, wherein the processor is programed to instruct the system to perform an iterative method comprising:

i) contacting the plurality of primed target nucleic acid sequences coupled to the at least one interior surface of the flow cell with a nucleotide composition to form a transient binding complex between the plurality of primed target nucleic acid sequences and a plurality of nucleotide moieties when a nucleotide moiety of the nucleotide composition is complementary to a nucleotide of the primed target nucleic acid sequence; and

ii) imaging the at least one interior surface of the flow cell using the multiplexed read-head to detect the transient binding complex and thereby determine the identity of the nucleotide of the primed target nucleic acid sequence.

76. The system of claim **75**, wherein the nucleotide composition comprises a conjugated polymer-nucleotide composition.

77. The system of claim **76**, wherein the conjugated polymer-nucleotide composition comprises a plurality of nucleotide moieties conjugated to a polymer core.

78. The system of claim **77**, wherein the plurality of nucleotide moieties comprises nucleotides, nucleotide analogs, or any combination thereof.

79. The system of claim **77** or claim **78**, wherein the plurality of nucleotide moieties comprises a plurality of identical nucleotide moieties.

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80. The system of any one of claims **75** to **79**, wherein the method is used to determine the identity of an N+1 or terminal nucleotide of a primer strand of the primed target nucleic acid sequence.

81. The system of any one of claims **75** to **80**, wherein prior to forming the transient binding complex the nucleotide composition lacks a polymerase.

82. The method of any one of claims **75** to **81**, wherein the plurality of primed target nucleic acid sequences is tethered to a first interior surface and a second interior surface of the flow cell, and wherein a first subset of the plurality of microfluorometers is configured to acquire wide-field images of different areas of the first interior surface of the flow cell, and a second subset of the plurality of microfluorometers is configured to acquire wide-field images of different areas of the second interior surface of the flow cell.

83. The system of any one of claims **75** to **82**, further comprising a translation stage configured to move the multiplexed read-head assembly in at least one direction parallel to the first and second sample planes.

84. The system of any one of claims **75** to **83**, wherein at least one microfluorimeter of the plurality of microfluorimeters is configured to acquire wide-field images having a field-of-view of at least 1 mm.

85. The system of any one of claims **75** to **84**, wherein at least one microfluorimeter of the plurality of microfluorimeters is configured to acquire wide-field images having a field-of-view of at least 1.5 mm.

86. The system of any one of claims **74** to **85**, wherein at least one of the microfluorometers further comprises a dedicated autofocus mechanism.

87. The system of claim **86**, wherein the autofocus mechanism for a first microfluorometer is configured to integrate data from an autofocus mechanism for a second microfluorometer, whereby the autofocus mechanisms for the first microfluorometer alters a focus of the first microfluorometer based on a focus position of the first microfluorometer and a focus position of the second microfluorometer.

88. The system of any one of claims **75** to **87**, wherein an individual microfluorometer of the plurality further comprises an objective, a beam splitter and a detector, wherein the beam splitter is positioned to direct excitation radiation from an excitation radiation source to the objective and to direct emission radiation from the objective to the detector.

89. The system of claim **88**, wherein at least one individual microfluorometer further comprises an individual excitation radiation source.

90. The system of claim **89**, wherein the excitation radiation source directs the excitation radiation to the objectives of two or more individual microfluorometers of the plurality such that the two or more individual microfluorometers share the excitation radiation source.

91. The system of any one of claims **88** to **90**, wherein two or more individual microfluorometers of the plurality further comprise or share at least two excitation radiation sources.

92. The system of any one of claims **88** to **91**, wherein the objectives of the individual microfluorometers of the plurality have a numerical aperture between 0.2 and 0.5.

93. The system of any one of claims **75** to **92**, wherein the microfluorometers of the plurality are configured to acquire images at a resolution sufficient to distinguish features that are less than 50 microns apart.

94. The system of any one of claims **82** to **93**, wherein the microfluorometers of the plurality are configured to have a depth-of-field that is less than the separation distance between the first and second interior surfaces of the flow cell.

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95. The system of any one of claims **82** to **94**, wherein the first subset of the plurality of microfluorometers is configured to acquire wide-field images at a first fluorescence emission wavelength and the second subset of the plurality of microfluorometers is configured to acquire wide field images at a second fluorescence emission wavelength.

96. A method of sequencing a nucleic acid molecule, the method comprising:

a) providing a surface; wherein the surface comprises:

i) a substrate;

ii) at least one hydrophilic polymer coating layer;

iii) a plurality of oligonucleotide molecules attached to at least one hydrophilic polymer coating layer; and

iv) at least one discrete region of said surface that comprises a plurality of clonally-amplified, sample nucleic acid molecules immobilized to said plurality of attached oligonucleotide molecules, wherein said plurality of immobilized clonally amplified sample nucleic acid molecules are present at distance less than $\kappa/(2*NA)$, wherein λ is the center wavelength of an excitation energy source and NA is the numerical aperture of an imaging system.

b) applying a stochastic photo-switching chemistry to said plurality of clonally amplified sample nucleic acid molecules at the same time to cause said plurality of clonally amplified sample nucleic acid molecules to fluoresce in on and off events in up to four different colors by stochastic photo-switching; and

c) detecting said on and off events in a color channel for each color in real-time as the on and off events are occurring for said plurality of clonally amplified sample nucleic acid molecules to determine an identify of a nucleotide of said clonally amplified sample nucleic acid molecule.

97. The method of claim **96**, wherein concentrations of reagents for said stochastic photo switching are sufficient such that the probability that an on event for a given nucleotide for a given clonally amplified sample nucleic acid molecule of said plurality of clonally amplified sample nucleic acid molecules will occur at the same time as an on event for a given nucleotide of a clonally amplified sample nucleic acid molecule adjacent to said given clonally amplified sample nucleic acid molecule is less than about 0.5%.

98. The method of claim **96**, further comprising, controlling a rate at which said on and off events occur to control a probability that an on event for a given nucleotide for a given clonally amplified sample nucleic acid molecule will occur at the same time as an on event for a nucleotide of a clonally amplified sample nucleic acid molecule adjacent to said given clonally amplified sample nucleic acid molecule.

99. The method of claim **98**, wherein controlling said rate at which said on and off events occur comprises adjusting concentrations of nucleotides and enzymes in said stochastic photo-switching chemistry.

100. The method of claim **96**, further comprising, determining whether an illumination intensity of a detection event in a color channel is greater than a predetermined threshold.

101. The method of claim **96**, further comprising, determining whether a spot size of a detection event in a color channel is greater than a predetermined threshold.

102. The method of claim **96**, wherein said at least one hydrophilic polymer coating layer comprises PEG.

103. The method of claim **96**, wherein detecting comprises acquiring an image of said surface, wherein said image exhibits a contrast to noise ratio (CNR) of at least 40.

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104. The method of claim **96**, wherein detecting comprises acquiring an image of said surface, wherein said image exhibits a contrast to noise ratio (CNR) of at least 60.

105. The method of claim **96**, wherein said substrate comprises glass.

106. The method of claim **96**, wherein said substrate comprises plastic.

107. The method of claim **96**, wherein said surface is positioned on the interior of a flow channel.

108. The method of claim **96**, wherein said at least one hydrophilic polymer layer comprises a branched hydrophilic polymer having at least 8 branches.

109. The method of claim **96**, wherein a background fluorescence intensity measured at a region of said surface that is laterally-displaced from said at least one discrete region is no more than 2× of the intensity measured at said at least one discrete region prior to said clonal amplification.

110. The method of claim **96**, wherein said sample nucleic acid molecules comprise single-stranded multimeric nucleic acid molecules comprising repeats of a regularly occurring monomer unit.

111. The method of claim **110**, wherein said single-stranded multimeric nucleic acid molecules are at least 10 kb in length.

112. The method of claim **110**, further comprising double-stranded monomeric copies of the regularly occurring monomer unit.

113. The method of claim **96**, wherein said surface comprises a first layer comprising a monolayer of polymer molecules tethered to a surface of said substrate; a second layer comprising polymer molecules tethered to said polymer molecules of said first layer; and a third layer comprising polymer molecules tethered to said polymer molecules of said second layer, wherein at least one layer comprises branched polymer molecules.

114. The method of claim **113**, wherein said third layer further comprises oligonucleotides tethered to said polymer molecules of said third layer.

115. The method of claim **114**, wherein said oligonucleotides tethered to said polymer molecules of said third layer are distributed at a plurality of depths throughout said third layer.

116. The method of claim **113**, further comprising a fourth layer comprising branched polymer molecules tethered to said polymer molecules of said third layer, and a fifth layer comprising polymer molecules tethered to said branched polymer molecules of said fourth layer.

117. The method of claim **116**, wherein said polymer molecules of said fifth layer further comprise oligonucleotides tethered to said polymer molecules of said fifth layer.

118. The method of claim **117**, wherein said oligonucleotides tethered to said polymer molecules of said fifth layer are distributed at a plurality of depths throughout said fifth layer.

119. The method of claim **96**, wherein said at least one hydrophilic polymer coating layer, comprises a molecule selected from the group consisting of polyethylene glycol (PEG), poly(vinyl alcohol) (PVA), poly(vinyl pyridine), poly(vinyl pyrrolidone) (PVP), poly(acrylic acid) (PAA), polyacrylamide, poly(N-isopropylacrylamide) (PNIPAM), poly(methyl methacrylate) (PMA), poly(2-hydroxyethyl methacrylate) (PHEMA), poly(oligo(ethylene glycol) methyl ether methacrylate) (POEGMA), polyglutamic acid (PGA), poly-lysine, poly-glucoside, streptavidin, and dextran.

While preferred embodiments of the present invention have been shown and described herein, it will be obvious to

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those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in any combination in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

What is claimed is:

1. A method for nucleic acid sequencing, said method comprising:

a) providing a flow cell comprising (i) a first surface comprising a first plurality of primed nucleic acid sequences coupled thereto and (ii) a second surface comprising a second plurality of primed nucleic acid sequences coupled thereto, wherein said second surface is axially displaced from said first surface along an optical path;

b) contacting said first plurality of primed nucleic acid sequences and said second plurality of primed nucleic acid sequences with (i) a plurality of nucleotide moieties comprising a plurality of fluorescent labels, and (ii) a polymerizing enzyme under conditions such that at least a subset of said plurality of nucleotide moieties couple to at least a subset of said first plurality of primed nucleic acid sequences and said second plurality of primed nucleic acid sequences; and

c) imaging said first surface and said second surface to detect a plurality of signals from a subset of said plurality of fluorescent labels, wherein said detecting comprises:

(i) illuminating said first surface and said second surface with a first light provided by a structured illumination system under a first set of illumination conditions to project a first plurality of fringes oriented in a specific direction on said first surface and said second surface;

(ii) capturing a first plurality of phase images of said first surface and said second surface;

(iii) illuminating said first surface and said second surface with a second light provided by said structured illumination system under a second set of illumination conditions to project a second plurality of fringes on said first surface and said second surface; and

(iv) capturing a second plurality of phase images of said first surface and said second surface illuminated with said second plurality of fringes.

2. The method of claim **1**, wherein:

(i) during said capturing of said first plurality of phase images in (c)(ii), positions of said first plurality of fringes are shifted on said first surface and said second surface;

(ii) said second plurality of fringes are angularly offset from said first plurality of fringes on said first surface and said second surface; and

(iii) during said capturing of said second plurality of phase images, positions of said second plurality of fringes are shifted on said first surface and said second surface.

3. The method of claim **1**, wherein a nucleotide moiety of said plurality of nucleotide moieties is conjugated to a polymer core to form a conjugated polymer-nucleotide.

4. The method of claim **3**, wherein said contacting in (b) comprises contacting said first plurality of primed nucleic

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acid sequences and said second plurality of primed nucleic acid sequences with said conjugated polymer-nucleotide to form a transient binding complex between at least one primed nucleic acid sequence of said first plurality of primed nucleic acid sequences and said second plurality of primed nucleic acid sequences, and a nucleotide moiety of said conjugated polymer-nucleotide, when said nucleotide moiety is complementary to a nucleotide of said at least one primed nucleic acid sequence.

5. The method of claim 4, wherein said imaging in (c) comprises detecting a plurality of signals from said transient binding complex.

6. The method of claim 1, wherein said first plurality of fringes and said second plurality of fringes are generated by a diffraction grating.

7. The method of claim 1, wherein said structured illumination system comprises a light source.

8. The method of claim 6, wherein said diffraction grating comprises a horizontal grating, vertical grating, or any combination thereof.

9. The method of claim 8, wherein said first surface is illuminated by said vertical grating and said second surface is illuminated by said horizontal grating.

10. The method of claim 6, wherein said diffraction grating comprises a transmissive phase diffraction grating.

11. The method of claim 1, wherein said second plurality of fringes is shifted by about 90 degrees from said first plurality of fringes.

12. The method of claim 1, wherein said structured illumination system comprises an optical phase modulator or phase shifter.

13. The method of claim 12, wherein said optical phase modulator or said phase shifter shifts said first plurality of fringes and second plurality of fringes by $\frac{1}{2}$, $\frac{1}{3}$, or $\frac{1}{4}$ of a pitch of said first plurality of fringes and said second plurality of fringes.

14. The method of claim 12, wherein said optical phase modulator comprises rotating optical phase plates actuated by rotatory actuators.

15. The method of claim 1, wherein said structured illumination system comprises one or more of:

- (1) a collimating optical element;
- (2) a polarizer; and
- (3) a diffraction grating.

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16. The method of claim 1, wherein said structured illumination system comprises a partially-silvered mirror that combines said first light and said second light.

17. The method of claim 1, wherein said first plurality of phase images or said second plurality of phase images comprise at least 1 angular orientation of said first plurality of fringes or said second plurality of fringes.

18. The method of claim 1, wherein said first plurality of phase images or said second plurality of phase images further comprise at least 1 phase shift of said first plurality of fringes or said second plurality of fringes.

19. The method of claim 1, wherein a nucleotide moiety of said at least said subset of said plurality of nucleotide moieties couples to a primed nucleic acid sequence of said at least said subset of said first plurality of primed nucleic acid sequences and said second plurality of primed nucleic acid sequences in (b) by incorporating said nucleotide moiety into said primed nucleic acid.

20. The method of claim 1, wherein:

(a) said structured illumination system comprises:

- (i) a light source;
- (ii) an optical phase modulator that rotates optical phase plates actuated by rotatory actuator;
- (iii) a phase shifter that shifts said first plurality of fringes and said second plurality of fringes by $\frac{1}{2}$, $\frac{1}{3}$, or $\frac{1}{4}$ of a pitch of said first plurality of fringes and said second plurality of fringes;
- (iv) a collimating optical element;
- (v) a polarizer;
- (vi) a diffraction grating that generates said first plurality of fringes and second plurality of fringes, wherein said diffraction grating comprises a horizontal grating, vertical grating, a transmissive phase diffraction grating, or any combination thereof, and wherein said second plurality of fringes is shifted by about 90 degrees from said first plurality of fringes; and

(vii) a partially-silvered mirror that combines said first light and said second light; and

(b) said first plurality of phase images or said second plurality of phase images comprises:

- (viii) at least 1 angular orientation of said first plurality of fringes or said second plurality of fringes; and
- (ix) at least 1 phase shift of said first plurality of fringes or said second plurality of fringes.

* * * * *

EXHIBIT 26

Native File Lodged with the Court

EXHIBIT 27

Native File Lodged with the Court

EXHIBIT 28

				Overview	Specifications	Pricing	Resources
750 million (3/4 scan)	12/17 hours	13/21 hours	15/27 hours	17/32 hours	19/43 hours		
500 million (1/2 scan)	12/15 hours	14/20 hours	17/24 hours	19/29 hours	24/39 hours		
250 million (1/4 scan)	11.5/14 hours	13/18 hours	15/22 hours	17/26 hours	21/34 hours		
125 million (1/8 scan)	11/13 hours	12.5/17 hours	14/21 hours	16/24 hours	20/31 hours		

Specifications

AVITI™ System

Performance Parameters	Flow cells: Two random access Read pairs PF/flow cell: ≥ 1 billion Maximum Read Length: 2 x 150 bp
Operating Environment	Temperature: 18-26 °C Altitude: < 2000 m Sound level: ≤ 62 db at 3.3 ft
Physical Dimensions	(H × W × D) 29.5 in × 37.6 in × 28.5 in Weight: 155.1 kg/342 lb
Crated Dimensions	(H × W × D) 48.6 in × 51 in × 35 in Weight: 245.9 kg/527 lb
Power Requirements	100-240 V at 50/60 Hz, 15 A, 550 W (average)
Operating System	Ubuntu Core 20.04 LTS
Safety & Compliance	NRTL certified IEC 61010-1:2010



Overview

Specifications

Pricing

Resources

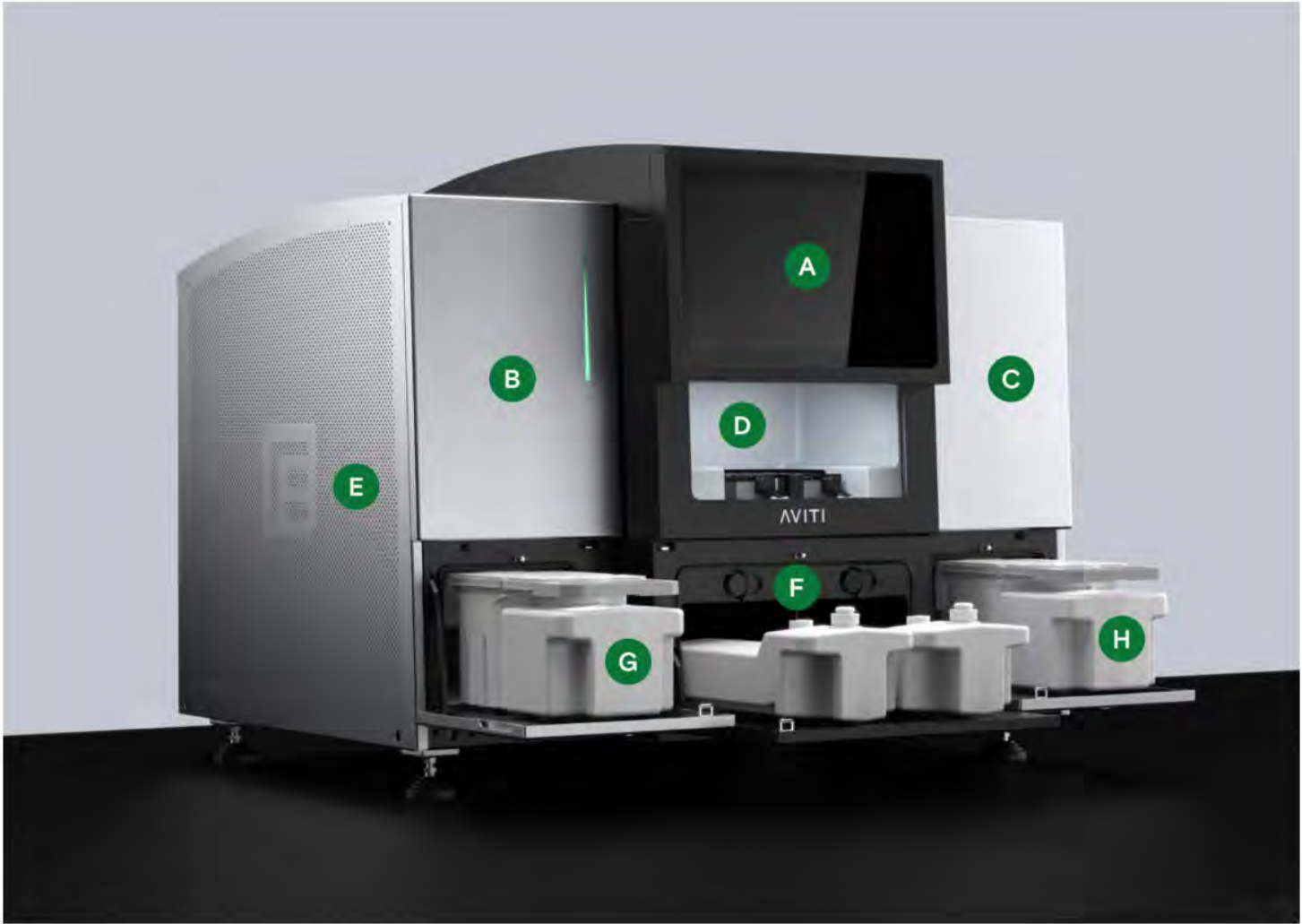
Safety & Compliance

NRTL certified IEC 61010-1:2010

FCC certified

Download AVITI™ System Specifications





Instrument Layout

The random access dual flow cell design is like having two sequencing instruments on your benchtop for the cost of one. Each side is independently controlled through the AVITI Operating Software. The intuitive touchscreen interface is positioned in the middle of the unit for convenient proximity to all its functional bays. The instrument also features electronic sensors for bar code identification of reagents for workflow guidance and error avoidance. Door lock sensors provide safety during operation.

Instrument Status Lights

A prominent indicator light on each side of the instrument communicates the instrument status so it is visible from the front-facing view at either side.

Parts

- A Touchscreen user interface for run setup
- B Fluidics pump bay side A
- C Fluidics pump bay side B
- D Flow cell nest bay
- E Ventilated housing
- F Two waste bottles, one per flow cell
- G Sequencing reagent cartridge side A
- H Sequencing reagent cartridge side B

Flow Cell Nest Bay Status Indicators

A button unlatches and opens the lid to a 40° angle. A wall encircles the loading area to guide placement and an LED light in front of each nest indicates status.

Resources

Brochure

View All Resources

Overview Specifications Pricing Resources

Instrument Status Lights

A prominent indicator light on each side of the instrument communicates the instrument status so it is visible from the front-facing view at either side.

New Connect Bay Status Indicators

A button unlatches and opens the lid to a 40° angle. A wall encircles the loading area to guide placement and an LED light in front of each nest indicates status.

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EXHIBIT 29

AVITI24 > Platform

AVITI24 System and Specifications

One integrated platform for single cell multiomics and sequencing

Multiomics

RNA, protein, & morphology profiled simultaneously

24 hr

Run times

2M cells

Profiled per run at subcellular resolution

20 cm²

Imaging area per run



Flexible

2 independent flow cells with 2 individually addressable lanes

Q50

Sequencing read quality Cloudbreak UltraQ

3B reads

Run output for 2 x 150 (available mid 2025)

<\$1

Per million reads

Powered by the flexibility of ABC sequencing, AVITI24 is the first and only platform to enable co-detection of RNA, protein, and morphology and NGS in one dual-sided run. With a first-of-its-kind onboard image processing system, AVITI24 seamlessly delivers industry-leading data quality. Independent run starts and ultra-fast run times provide unparalleled flexibility to accelerate your discovery.

Specifications

Sequencing Specifications

Read Count ^a	High output:	1.5 B reads per flow cell
	Medium output:	750 M reads per flow cell
	Low output:	375 M reads per flow cell
Accuracy	> 90% Q30 with 2 x 150 and 2 x 75 cycles	
	> 85% Q30 with 2 x 300 cycles	
	> 70% Q50 with Cloudbreak UltraQ™ kits ^c	
Inputs	Direct loading of linear libraries with Cloudbreak Freestyle™ kits	
Run Time ^b	≤ 24 hours 2 x 75 cycles	
	≤ 38 hours 2 x 150 cycles	
	≤ 60 hours 2 x 300 cycles	

^a Increased output available Mid 2025. Performance metrics are based on sequencing Element libraries. Actual results might differ due to library type and preparation methods.

^b Individually addressable lanes and custom recipes can extend run times.

^c Based on Elevate™ libraries and specific run parameters.

Cytoprofiling Specifications

Analytes	RNA, protein, morphology
	100 bp in situ RNA sequencing in 2025
Plex	RNA: 350 targets
	Protein: 50 targets
	Morphology: 6 markers
Content	MAP Kinase Cell Cycle and Apoptosis
	Immunology, neuroscience, and custom panels in 2025
Imaging	< 250 nm subcellular spatial resolution with multi-feature cell segmentation
Inputs	Adherent cells

Cell suspensions in 2025

Sensitivity	1 M mean counts detected per mm ²
Throughput	Up to 1 M cells with 10 cm ² area per flow cell Two flow cells per run
Format	12 wells (0.5 cm ² /well) 1 well (10 cm ² /well)
Run Time	24 hours ^a
Sample Prep	45 minutes

^a Run times based on a single 12 well run with software update available in early 2025

System Specifications

Instrument Configuration	Dual flow cells AVITI Operating Software Ubuntu Core 20.04 LTS operating system
Operating Environment	Temperature: 18°C to 26°C Elevation: < 2000 m Sound level: ≤ 62 db at 3.3 ft
Instrument Dimensions	(H x W x D) 29.5 in x 37.6 in x 28.5 in Weight: 155.1 kg/342 lb
Power Requirements	100-240 VAC at 50/60 Hz 15 A, 550 W (average)

Ordering Information

Element AVITI24 System	880-00004
Element AVITI24 Upgrade	895-00060

Overview Platform CytoProfiling Resources

Resources



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Job Function 

Cancer 

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EXHIBIT 30

Native File Lodged with the Court

EXHIBIT 31

Native File Lodged with the Court